Annals
of the
Missouri Botanical Garden

Vol. 33 SEPTEMBER, 1946 No. 3

A CYTOLOGICAL STUDY OF YEAST *(SACCHAROMYCES CEREVISIAE)*[1]

LILLIAN NAGEL

Associate Professor of Biology, Harris Teachers College, St. Louis
Formerly Instructor in the Henry Shaw School of Botany of Washington University

INTRODUCTION

A century ago Nägeli (1844) first described the yeast cell as having "a little nucleus of whitish mucus, lying on the membrane." From that time to this the cytology of yeast has been a subject of controversy. Extensive bibliographies and discussions of the earlier literature may be found in Wager ('98), Wager and Peniston ('10), Guilliermond ('20), Moore ('33), and Badian ('37). Brandt ('41) has reviewed the more recent literature, as well as given the results and interpretations of his own extensive investigations. Recently Lindegren ('45) and Subramaniam and Ranganathan ('45, '46a, '46b) have published differing interpretations of nuclear organization in yeast.

In spite of the great amount of work which has been done on the cytology of yeasts, there is still no general agreement (Table VI) among students of the subject even on fundamental points. There are several reasons for this lack of agreement: (1) The most important is the small size of the yeast cell which makes accurate observation of details extremely difficult and causes interpretation to be more or less speculative. (2) The use of only one or two stain techniques by many investigators has increased the confusion because interpretations are based on incomplete information. (3) Until the work of Winge and his associates ('35, '37) investigators were handicapped by lack of understanding of the life cycle of yeasts. (4) Not until the recent work of Lindegren and Lindegren ('44) was it possible for a cytologist to secure adequate sporulating material.

The present work was undertaken with the hope that additional information could be gained concerning the structure of the yeast cell through the thorough and methodical use of a variety of techniques on the excellent material available from the Lindegren laboratory.

[1]An investigation carried out in the graduate laboratory of the Henry Shaw School of Botany of Washington University, assisted by a grant from Anheuser-Busch Inc., and submitted as a thesis in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

MATERIAL

Saccharomyces cerevisiae was used in all of the work reported in this paper except as noted below. The cultures were secured from the Lindegrens, and all culture designations refer to their material. Most work was done with the diploids LK, with the hybrids EFF, 30 x 20 cc., and with BC20—, and several similar haploids. EFF is a hybrid of three commercial baking yeasts; LK is isolated from a Canadian pressed yeast. The haploid BC20— is a single ascospore isolate from a strain studied by the Lindegrens as were the other haploids used.

While working on the identification of wild yeasts found on native fruits obtained from the Missouri Botanical Garden Arboretum, an especially large one which showed peculiar multiple budding was isolated. It is probably an undescribed species of *Syringospora* or a related genus. Limited cytological observations were made on it for comparison with *Saccharomyces cerevisiae*. A few observations were also made on a species of *Cryptococcus* (?), another wild yeast.

METHODS

In so far as possible all techniques and observations were carried out with all material used; that is, a general systematic survey was attempted. Parallel observations of living material were also made.

Culture Methods.—Budding cells of various diploid cultures were sometimes obtained from yield tests in the Lindegren Laboratory (Lindegren and Lindegren, '43). Both haploids and diploids were also cultured in liquid nutrient medium

TABLE I

PROCEDURE FOR SECURING SPORULATION
(LINDEGREN AND LINDEGREN, '44)

TIME	MEDIUM			TECHNIQUE
	Kind	Formula	Method of Preparation	
48–72 hrs.	Presporulating	Beet (leaves) extract ....10 cc. Beet (roots) extract.....20 cc. Apricot juice .....35 cc. Grape juice .....16½ cc. Yeast (dried) .....2 gm. Glycerin .....2½ gm. Agar .....3 gm. $CaCO_3$ .....1 gm. Water—to give 100 cc. final volume	Steam mixture 10 minutes. Tube and autoclave. Slant.	Streak on slant; incubate at 25° C. (room temp.). After 2–3 days pour 1 cc. sterile water over yeast. Stir to make thick suspension of cells. Take up in sterile pipette.
16–48 hrs.	Gypsum slant	Water .....100 cc. Plaster of Paris .....100 gm.	Mix, pour, and slant rapidly. Dry 24 hours at 50° C. Autoclave.	Pour over upper part of gypsum slant. Pipette 3–4 cc. sterile water containing enough acetic acid to bring it to pH 4.0 into lower half of slant. Incubate at 25° C.

and on nutrient agar slants. Little work was done with copulating yeast cells or with old or starved cultures.

Material for the study of stages of sporulation was cultured in accordance with the method developed by the Lindegrens on medium supplied by them (Table I). The diploid strains LK and EFF were usually used because they sporulate well.

Smear Technique.—All permanent preparations were made as smears on cover glasses and stained in cover glass Coplin jars (cover glass wells of Thomas & Co.). The yeast cells were stuck to the cover glass with a thin coating of Mayer's albumen fixative or applied directly to a thoroughly cleaned cover glass. The cover glasses with the layer of albumen were generally heated gently over an alcohol burner "until a gray smoke is given off" as suggested by La Cour ('41) before applying the cells. Many more cells remained on the cover glass if fixative was applied, but with some techniques the albumen tended to stain and if drying occurred artifacts frequently appeared. Many cells were lost in succeeding operations if applied directly to the cover glass.

For applying the yeast to the cover slip a micropipette was found useful. An ordinary glass dropper was heated just above the narrow end, drawn out thin, cut off to a length of seven or eight inches, and the rubber bulb replaced. A very small drop of water was placed on the cover glass with the micropipette and cells were added from a slant with a sterile loop; in other cases a droplet of cells was pipetted onto the cover slip from a suspension. The cells were spread evenly by passing the thin flexible end of the micropipette across the surface of the cover glass. This was done rapidly to avoid drying, which causes severe distortion of the cells. The cover glasses were immediately floated cell-side down on the surface of the fixing solution in a Petri dish. If long fixation was required, they were transferred after about fifteen minutes to a cover glass Coplin jar for convenient storage.

Fixation and Staining Procedures.—Many different fixations were tried, and the types which apparently caused least shrinkage, distortion of the vacuole, and granulation of the cytoplasm were generally used (Table II). Drying, even after fixation, tended to cause shrinkage and distortion. Good fixation was apparently related in part to the condition of the cells; fresh, actively growing cells showed better fixation than old cells from giant colonies, and actively budding cells better than the highly granular sporulating cells. None of the methods used was completely satisfactory.

The general staining procedures are outlined in Table III. A further discussion of outstanding points is given in the following section of the paper.

TABLE II

FIXATION*

Fixative	Formula and/or Modifications	Time	Evaluation
Mercuric chloride	Saturated aqueous $HgCl_2$ + 0.5–1.0% glacial acetic acid	½–2 hrs.	Most satisfactory general fixation
Iodine-formalin-acetic acid	1% iodine in 1% KI..20.0 cc. Formalin 40% ..... 4.0 cc. Glacial acetic acid ..... 0.5 cc. Distilled water .....5–10.0 cc.	12–24 hrs.	Good with Giemsa technique (2, Table III)
Navashin	Plant tissue formula (Lee, '37) "Craf" Modification (Johansen, '40)	1–12 hrs.	Fair. Distortion of vacuole common
Osmic acid vapor	Vapor from 1% osmic acid solution	3–5 min.	Fair. Cytoplasm granular, vacuole often distorted or invisible
Picric acid	Saturated in distilled water or 70% alcohol	½–12 hrs.	Fair with methylene blue-eosin technique (7, Table III)

*Other fixations tried but not used because of distortion, shrinkage, and/or granulation of the cytoplasm were Carnoy, Carnoy-Lebrun, Perenyi, Flemming's weak solution, Uranium fixative (Semmens, '42), Picro-formol-acetic acid mixtures, glacial acetic acid vapor, chrome-Bowen-urea fluid, and alcohol both alone and in various combinations.

TABLE III

STAINING METHODS EMPLOYED IN THE STUDY OF THE YEAST CELL*

Stain	Usual fixation	Staining procedure	Remarks
1. Feulgen (Johansen, '40; de Tomasi, '36; Coleman, '38)	Mercuric chloride Navashin (Unnecessary to wash out fixing solution before hydrolysis—Bensley & Bensley, '38)	1. Hydrolyze 7–8 min, in 1 *N* HCl at 60° C. (Hillary, '39). 2. Stain 4–5 hrs. in Feulgen. 3. Wash 10 min. in each of three HCl-$K_2S_2O_5$ baths.	Feulgen positive body present in all cells. Cytoplasm clear and colorless.
2. Robinow's bacterial Giemsa (Robinow, '42; Dubos, '45)	Mercuric chloride Iodine Osmic vapor	1. Hydrolyze in 1 *N* HCl at 60° C. 7–10 min. 2. Stain in Giemsa (1 drop stain per ml. dilute buffer— pH 6.9–7.0) 15–45 min. 3. Pass through acetone-xylol mixtures as follows: 20:1 ..... 4–30 sec. 14:6 .....10–30 sec. 6:14 .....10–15 sec. Pure xylol .....10–15 min.	Very good; apparently stains Feulgen positive body and gives stronger contrast and differentiation.

TABLE III (continued)

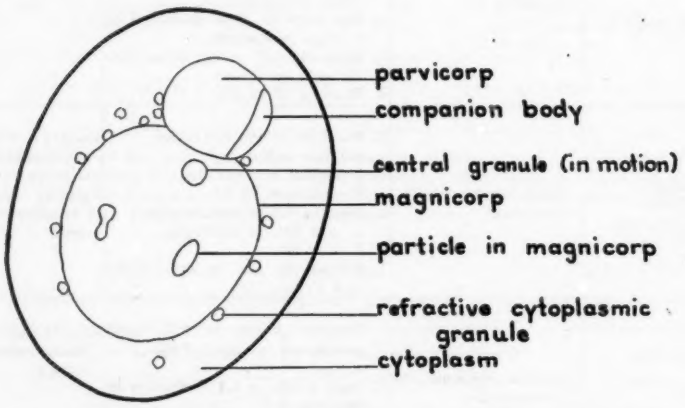
3. Methyl violet (Johansen, '40)	Navashin	1. Stain in 1% aqueous methyl violet 10 min. 2. Pass through: a. 0.5% picric acid in 70% alcohol 10 sec. b. 0.5% picric acid in 95% alcohol 10 sec. c. 95% alcohol (plus 4 drops NH_4OH per 100 cc.) 15 sec. d. 100% alcohol 10–15 sec. e. Clove oil 30–45 sec.	Results only fair; nothing added to those from other techniques.
4. Flemming's triple stain (Johansen, '40)	Navashin	1. Mordant 24 hrs. in 1% osmic acid in 2% chromic. 2. Stain in standard safranin solution 24 hrs. 3. Stain in 1% aqueous crystal violet 10–15 min. 4. Dip twice in 95% alcohol, 3 or 4 times in absolute. 5. Drop sat. sol. orange G in clove oil on c. g. 10 sec. 6. Wash in clove oil.	Results poor; added nothing to hematoxylin; could probably be improved by modification.
5. Heidenhain's hematoxylin (Johansen, '40; Lee, '37)	Mercuric chloride Navashin	1. Mordant in 4% ferric ammonium sulfate. Procedure a. 6–18 hrs. Procedure b. 1 hr. 2. Stain in 0.5% hematoxylin. a. 6–8 hrs. or overnight. b. 2–3 hrs. 3. Destain in sat. aqueous picric acid (Tuan, '30).	Shorter mordanting, staining, and destaining gave different picture from long. Cytoplasm tends to stain.
6. Brazilin	Same as Heidenhain's hematoxylin	1. Mordant 6 hrs. in 1% ferric ammonium sulfate in 70% alcohol. 2. Stain 6 hrs. in 0.5% Brazilin in 70% alcohol. 3. Destain in sat. picric in 70% alcohol.	Results similar to Heidenhain's hematoxylin.
7. Methylene blue-eosin (Badian, '37)	Picric acid Navashin Mercuric chloride	1. Overstain in 1% aqueous methylene blue 24–48 hrs. 2. Differentiate very slowly with dilute eosin (0.5% or less).	Results uncertain. Tends to lose stain in alcohol.
8. Aceto-, lacto-, and propionic carmine, orcein, Bismark brown	Alcohol-acetic acid. Also various mordants and pretreatments were used without success.	All combinations of the acids and stains in various concentrations were tried—heated and unheated, fresh and after standing. No permanent slides prepared.	Generally unsatisfactory. Aceto-orcein and propionic carmine usually some better than other combinations.

*All preparations were run up through alcohol series into xylol and mounted in balsam unless otherwise stated. Usual washing or rinsing steps not listed.

OBSERVATIONS

INTRODUCTION AND TERMINOLOGY

As mentioned in the Introduction there is as yet no general agreement among yeast cytologists even as to fundamentals. The body most frequently referred to as the nucleus (Guilliermond, '10, '20; Badian, '37; Beams, Zell, and Sulkin, '40) is called the centriole by another school (Lindegren, '45) and the nucleolus by a third (Wager and Peniston, '10). In the following observations new terms which are purely descriptive and have no previous connotation have accordingly been chosen in order to present a picture of results, independent of interpretations of nuclear organization in yeast. The term "parvicorp" (small body) will be used (text-fig. 1) to indicate that part of the yeast cell frequently designated as the



Text-fig. 1. Diagram of the yeast cell with parts labeled according to the terminology used in this paper.

nucleus. The term "magnicorp" (large body) will be used to designate the part usually called the vacuole, but also named the nucleus (Janssens and Leblanc, '98) and the nuclear vacuole (Wager and Peniston, '10; Lindegren, '45). A diagrammatic representation of these parts is shown in text-fig. 1.

Descriptions of budding material precede those of sporulating material. The results for each technique are given separately and in the order listed in Table III. The drawings on the plates follow the observations of the stained slides as closely as possible. All observations were made with a binocular microscope equipped with a 90 x apochromatic objective and 15 x compensating oculars.

SACCHAROMYCES CEREVISIAE
STUDIES OF BUDDING CULTURES

Feulgen Technique.—In all cells except small buds the parvicorp is at least faintly Feulgen positive (pl. 10). However, not all of the parvicorps exhibit the same color depth; a few are small and stain much more strongly than the larger ones. In some cells the parvicorp is evenly colored throughout, in others there is apparent variation in color intensity (pl. 10, figs. 4, 5, 11, 13). The buds often lack the parvicorp, but in that case it is frequently dividing in the mother cell or has finished its division or may not have started to divide, in which event it is commonly larger than usual (pl. 10, figs. 1–15). The division may be completed in the mother cell and the parvicorp then pass into the bud, but in many strains it more often appears to divide directly into the bud. In actively growing cultures the parvicorp is usually located proximal to the small bud and divides directly into it (Lindegren, '45). Where the budding is not quite so rapid the Feulgen positive body may be located between two parts of the magnicorp or even opposite the bud. After the buds acquire parvicorps the latter usually assume a distal position in mother and daughter cells (pl. 10, fig. 16). The parvicorp may vary in shape from round to oval, may be crescent-shaped, irregular in outline, or divided into two bodies, as noted earlier by Margolena ('32), Winge ('35), Badian ('37), and others. These bodies have often been interpreted as chromosomes (Table VI). Conventional mitotic figures were not seen in the dividing Feulgen-stained bodies of *Saccharomyces cerevisiae.* The Feulgen stain is faint and the parvicorp small, so that details are somewhat speculative.

The magnicorp, which was unstained but generally quite clear in outline, showed no Feulgen positive material with the procedure used. A discussion of the magnicorp will be deferred to the sections on methylene blue-eosin and Heidenhain's hematoxylin in which its structure is more readily observed. However, it might be mentioned here that the parvicorp almost universally lies in contact with the magnicorp.

Robinow's Giemsa Technique.—The use of this technique was suggested by La Cour (personal communication) after a number of others were found inadequate. The Robinow Giemsa bacterial stain ('42; Dubos, '45, with appendix by Robinow) was used with the following minor modifications. Osmic acid was slightly less satisfactory than mercuric chloride or iodine-formol-acetic acid as a fixation. Samples of stain were obtained from three sources and were found to vary somewhat in their staining ability. Destaining was not always even. Insufficiently destained and over-destained cells sometimes occurred on the same slide probably due to inadequate pH control. Difficulty with fading was overcome by controlling the pH of the various solutions, especially the balsam which was neutralized with sodium bicarbonate following the directions in Lee (9th edition, '28) and by storing the finished slides in darkness. The better slides produced by this method were the most satisfactory for observation of the parvicorp. However, good results were obtained only with the correct balance of all variables.

Giemsa stains the parvicorp in much the same manner as the Feulgen reaction but gives a much deeper color that shows more internal differentiation and greater contrast with the cytoplasm. Because of this sharpness of contrast the irregularity of the outline of many of the parvicorps is much more apparent than with Feulgen. Although many of the cells show parvicorps with relatively regular outlines more of those with irregular outlines are illustrated in pl. 11 in order to indicate the kind and degree of variation observed. The difference in color intensity within the stained bodies was often quite marked, resembling somewhat the chromocenters of higher plants. One or two small extrusions or appendages were often found. In older agar slant cultures, apparently aberrant divisions of the parvicorps without consequent division of the cell seemed to occur, giving such forms as shown in pl. 13, figs. 92, 93, 95–97. Haploid cells and their parvicorps were smaller than diploid, but measurements of the parvicorps were not attempted because their size variation in different stages of cell division made exact comparisons of doubtful value. Division of the parvicorp appeared much the same as with the Feulgen technique. A few of the parvicorps exhibited the bipartite structure noted in the preceding technique but the phenomenon seemed less in evidence, especially in the haploids.

The magnicorp was generally not visible with this technique, but when it was its relation with the parvicorp was usually clear and unquestionable. In these cells it lay in direct contact with the parvicorp as described above (pl. 11, figs. 30, 40, 42, 46). In a few cases various other relationships between these two bodies were observed, and several of these are illustrated (pl. 11, figs. 45, 47; pl. 13, figs. 96, 97). In these exceptional cells, however, the definition of the two bodies was somewhat obscure.

Methyl Violet and Flemming's Triple Stain.—Johansen's methyl violet staining method was used without appreciable change. Methods developed by Smith ('34), Newton (Johansen, '40), and Hancock ('42) did not give good results but would probably prove satisfactory if time were taken to modify them.

The parvicorp generally stained in much the same manner as with Feulgen (pl. 12, figs. 48–60), but sometimes only part of it retained the stain, the slides thus resembling certain Heidenhain's hematoxylin slides (pl. 12, figs. 51, 53, 56). The magnicorp showed no particles or stained structures. Similar but less certain results were obtained with Flemming's triple stain (pl. 12, figs. 61, 62). As these staining procedures provided little additional information to that acquired from other techniques and as they were more troublesome to carry out, they were not used extensively.

Heidenhain's Hematoxylin and Brazilin.—After long mordanting and staining with Heidenhain's hematoxylin (5a, Table III) the entire parvicorp remained black (pl. 13, figs. 72–81). With the short staining procedure (5b, Table III) usually a black area designated here as the companion body (text-fig. 1) stained at one place and the remainder of the parvicorp was lighter than the surrounding cyto-

plasm (pl. 13, figs. 82–89). This differentiation resembled that shown in Wager and Peniston's figures of the "chromatin patch" except that the "peripheral layer of chromatin" was not generally apparent, nor did the companion body exhibit as great a diversity of shape as the authors illustrate (Wager and Peniston, '10, pl. 16, figs. 53–80). This same phenomenon shows to some extent with Brazilin and methyl violet techniques but is not apparent with any of the others that were used, although identical fixations were frequently employed.

In either budding or resting cells this companion body was often double and occurred in any one of several positions, probably due to both the orientation under the microscope and the condition and stage of development of the cell. Division of the companion body at mitosis is difficult to follow even in slides of actively budding cultures. It seems to elongate greatly and divide by thinning in the center (pl. 13, figs. 83, 89). In the non-budding cell the parvicorp appears to have a very regular outline; in the budding cell it is not distinct as the contrast between cytoplasm and parvicorp is not great. With the longer staining procedure results are similar to those of the Feulgen and Giemsa techniques, but no differentiation within the parvicorp is visible, the boundary is usually regular, and no extrusions or appendages are apparent. The parvicorp may destain in various unusual patterns, especially in old or poorly nourished cells, and give rise to figures such as pl. 13, fig. 76, which, although they may resemble conventional mitotic stages, probably do not represent a division stage at all. Because the cytoplasm sometimes destains irregularly, results are difficult to interpret. Therefore, in spite of the recent work of Subramaniam and Ranganathan ('45), the author does not consider this technique one of the most satisfactory for the study of yeast cytology, certainly not the technique to use as the sole basis of interpretation.

The magnicorp appears clear and optically empty in budding material. However, if it is shrunken or distorted in fixation, as sometimes happens, the folds tend to retain the stain and give the appearance of strands passing over the surface (pl. 13, fig. 73). In well-expanded and preserved magnicorps this phenomenon was not apparent. Wager and Peniston reported a "chromatin network" over the surface of the magnicorp as a regular cell component, possibly because they regularly dried their preparations after fixation. There are times when denser strands of cytoplasm seem to radiate from the parvicorp and may or may not extend out over the magnicorp depending upon the relative positions of the two cell bodies (pl. 13, figs. 77, 78). The early entrance of the magnicorp into the bud is especially apparent with this stain when it follows mercuric chloride fixation. Subramaniam and Ranganathan have used a Carnoy fixing solution which usually leaves the magnicorp invisible and the relative positions of cell components uncertain.

Brazilin gives results similar to Heidenhain's hematoxylin, staining either the entire parvicorp or only the companion body depending upon the length of staining and destaining (pl. 12, figs. 63–71). With the shorter procedure the parvi-

corp sometimes shows shadowy material extending from the companion body (pl. 12, fig. 65).

Methylene Blue-Eosin Technique.—The Giemsa-eosin technique of Badian ('37) was modified by substituting methylene blue for Giemsa. It can be said of it also: "L'application de cette méthode de différenciation aux Levures n'est pas toujours facile—" [Badian, '37, p. 64]. Staining and differentiation of the cells in bulk in a centrifuge tube were more easily controlled than on the cover glasses although both methods were used.

Certain particles or granules in the yeast cell stained a brilliant blue-red when the differentiation was carried just far enough; further differentiation in eosin left the entire cell a faint, even pink. The position, number, and size of these particles apparently depended in part on physiological conditions, in part on fixation. In a 48-hour unshaken broth culture the stained bodies, if present, were found within the magnicorp (pl. 14, figs. 98–107); in a similar culture that was shaken and crowded colored particles appeared to lie at the periphery of the magnicorp or occasionally in the cytoplasm (pl. 14, figs. 109–112). In the unshaken culture, the parvicorp stained faint pink in the blue cytoplasm and was clearly visible in 98 per cent of the cells, but little or no differentiation was noted. The outline was quite regular and clear. In this same culture approximately 85 per cent of the yeast cells contained no particles at all in the magnicorp; the rest contained one or more of various sizes and shapes (Table IV). In the shaken culture the

TABLE IV

NUMBER OF PARTICLES PER CELL IN THE MAGNICORP OF YEAST CELLS STAINED WITH METHYLENE BLUE-EOSIN TECHNIQUE (100 CELLS SELECTED AT RANDOM).

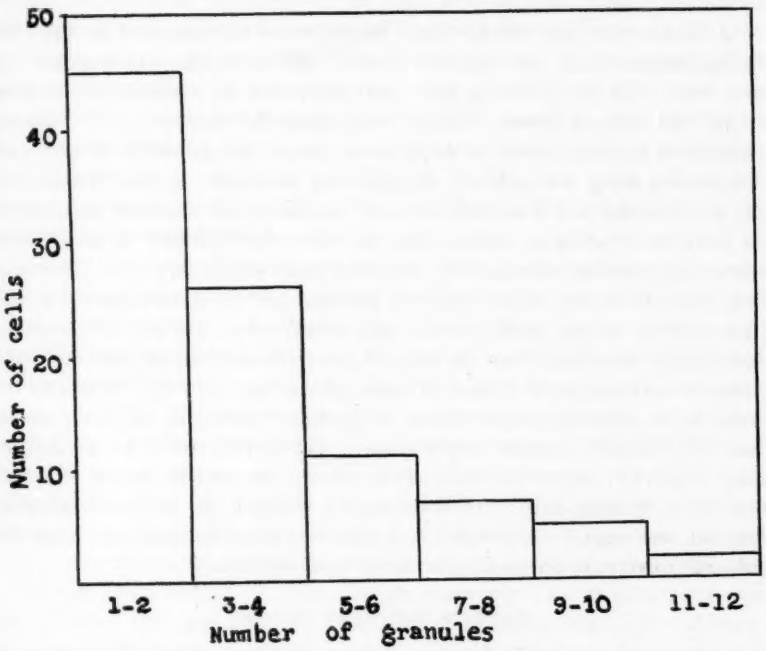
Number of particles per cell	0	1	2	3	4	5	6	7	8	9	10 or more
Number of cells	85	4	2	2	2	—	1	1	—	1	2

particles were exceedingly numerous and were present in most cells where differentiation was not carried too far. The parvicorp was not visible in these cells.

Granule number per cell was counted in 100 granule-containing cells (15 per cent of the total cell number). The results are diagrammed in text-fig. 2. Most of the cells in this culture contained few particles which at times appeared irregular in outline as if they were possibly aggregates, but the total amount of material in the magnicorps was not uniform.

In a few instances the particles appeared paired (Table V). The pairs of granules were similar in staining reaction, size, and shape. At times they occurred as separate particles; at times the two were joined in dumbbell ("diplokokken ähnlich", Henneberg, '16) or V-shaped masses with the two ends alike in shape and size. Rarely all of the particles of a cell appeared paired (pl. 14, fig. 107);

more often there were only one or two such pairs, the other particles not showing this condition. The number of pairs of granules was not constant. Due to the position and arrangement of granules in some cells it was not always possible to be certain whether or not they were paired. These are listed in Table V as "Possibly paired."



Text-fig. 2. Number of granules per cell in the magnicorps of 100 granule-containing cells.

TABLE V

PER CENT OF YEAST CELLS SHOWING PAIRING OF THE PARTICLES IN THE MAGNICORP WHEN STAINED WITH METHYLENE BLUE-EOSIN.

	% of granule-containing cells	% of total number of cells in culture
No apparent pairing	82.+	12.+
One or more pairs present	9.—	1.
Possibly paired	9.	1.+

Actively budding yeast from a yield test (4 hour 20 minute shaken culture) showed very faintly differentiated parvicorps and a few cells which contained as many as thirty to fifty stained particles. The latter were in or at the periphery of the magnicorp (pl. 14, fig. 108) and in several instances were passing into the bud. Due to the large number of somewhat similar granules in the cells pairing could not be determined.

Aceto-Carmine and Related Smear Techniques.—As mentioned in Table III, various concentrations and mixtures of acetic acid, lactic acid, and propionic acid were tried with the following dyes used singly and in combination: carmine, orcein, and Bismark brown. Results were generally unsatisfactory. Granular cytoplasmic material stained so deeply as to obscure the parvicorp in most cases and the magnicorp was generally shrunken and distorted. Various fixations beside acetic-alcohol and a number of other treatments and mordants suggested in the literature (Darlington and La Cour, '42) were tried with little success. Aceto-orcein and propionic carmine were somewhat more satisfactory than other solutions. Gentle heating helped bring out contrast, and the slides improved a little after standing for two to five days at room temperature. The cells which stained most clearly were those from the edge of giant colonies two to four weeks old, grown on nutrient agar in a moist chamber (pl. 14, figs. 113–119). Probably this is due to the relatively small amount of granular material in the cytoplasm of these cells (author's research, unpublished). The parvicorp may be rounded, or, more frequently, somewhat star-shaped, or may occasionally appear bipartite, especially in dividing cells. The magnicorp (if visible at all) is usually shrunken, distorted, and empty. Although much time was spent in trying to adapt this technique to yeast, it was finally abandoned as unsatisfactory.

Studies of Sporulating Cultures

Feulgen and Giemsa Techniques.—Because of the similarity of results with these techniques, they are considered together. In any sporulating culture only a few cells were in meiotic division at any one time, and the extremely small number found in certain stages would indicate that at least parts of the process are very rapid. Preceding spore formation, the parvicorp becomes approximately twice its normal diameter, appears filamentous, and resembles the prophase of the conventional meiotic division as closely as could be expected in anything as small and unique as a yeast cell (pl. 14, figs. 123–127; pl. 15, figs. 128–130, 141–144). This phase appears to be of long duration as it is quite common in slides of sporulating cultures. The next steps in the division are not too certain and probably proceed with great rapidity as few instances were found that could be definitely assigned to this phase in the many slides that were examined. Probably such stages as pl. 15, figs. 131, 145, 146, 149, 150 belong in this category. Very

rarely two parvicorps lying beside or above each other were found toward the center of the cell (pl. 15, fig. 151; pl. 16, figs. 173, 174). While this position might be the logical expectation, its extreme rarity leads one to believe that the second division normally follows the first so rapidly that the stage with two parvicorps may not actually become organized in most cells. The second division appeared to start near the center of the cell. Two elongate, slightly dumbbell-shaped masses were formed, the ends of which passed toward the periphery of the cell, usually toward opposite poles. A gradual thickening of the extremities of the masses occurred at the expense of the center (pl. 15, figs. 132–136; pl. 16, figs. 152–158). Such division figures were relatively common. Cells with elongate parvicorps which appeared bipartite or possibly four-partite were also relatively common in all sporulating material (pl. 15, figs. 147, 148). These may represent a stage which either follows the more diffuse early stage or might be the beginning of the second division. Instances of a single parvicorp at each pole of the cell were not positively ascertained; careful study of apparent examples usually showed that at least at one pole one body could be resolved above the other, and that the second division had actually taken place. When division is first completed, the parvicorps usually lie at the periphery of the cell close to the wall and the spore plasm lying between them is frequently more dense than the epiplasm[1] (pl. 16, fig. 160). The organization of the ascospores seems to occur in a manner similar to that of other Ascomycetes. Apparently the spore is delimited by being cut out by rays extending from the parvicorp. At times one or two of the ascospores develop more rapidly than the others. Ray-like strands of cytoplasm frequently extend out from the parvicorp in the fully formed ascospore. If only two or three ascospores develop, the other parvicorps can be observed lying free in the ascus (pl. 15, figs. 139, 140). Single spores with two or more parvicorps were not seen. Old agar slants of diploid cells, some sporulating, showed aberrant divisions in which the parvicorp apparently divided without subsequent cell division. In some of these cells the parvicorp was divided into separate particles (pl. 13, fig. 97).

Meiotic division of the magnicorp could not be followed in either technique. Although this body is generally visible in budding material stained with Feulgen, it was imperfectly visible, if at all, in sporulating cells (except in the fully developed ascospores where it again appeared empty).

Notes On Other Techniques.—Aceto-orcein was unsuccessful as a stain for the division figures of spore formation because of the deeply staining, granular cytoplasm. However, the formation of the ascospores was similar to that observed with Giemsa and in the fully developed ascospores an empty magnicorp was generally visible (pl. 17, figs. 193, 194).

[1] In Ascomycetes, the cytoplasmic contents of the ascus not used in spore formation.

Methylene blue-eosin was not especially useful as a stain for sporulating material, nor did the conventional volutin reaction give an enlightening picture of the magnicorp and its particles in meiosis. With both techniques, if magnicorps were visible, stained particles in them were few (exceptionally there were many particles or none). No regular organization of nagnicorp and its contents was ascertained.

Slides stained by the longer procedure for Heidenhain's hematoxylin (5a, Table III) or by Brazilin gave much the same results as Giemsa and Feulgen, but less distinct due to the retention of stain by the granular cytoplasm (pl. 16, figs. 165–175). If stained by the shorter method (5b, Table III), the results were so variable as to be uninterpretable and the companion body could not be followed. The magnicorp, though evident in budding and resting cells with these techniques, could not be followed in sporulating material.

Studies of Living and Supra-vitally Stained Cultures

Comparative studies on living cells of cultures were made whenever slides were prepared. In budding cells the parvicorp was only rarely visible in living cells, although its position could often be determined by a slight invagination of the magnicorp. The latter body could almost always be seen except in certain small, very dense cells where it was probably obscured by the highly refractive surrounding cytoplasm. It occurred usually as one body, occasionally as two, more rarely as several to many, depending on the age, nutrition, aeration, and other cultural conditions of the cells. At times one to several particles could be seen moving in the magnicorp, and occasionally these appeared to be paired.

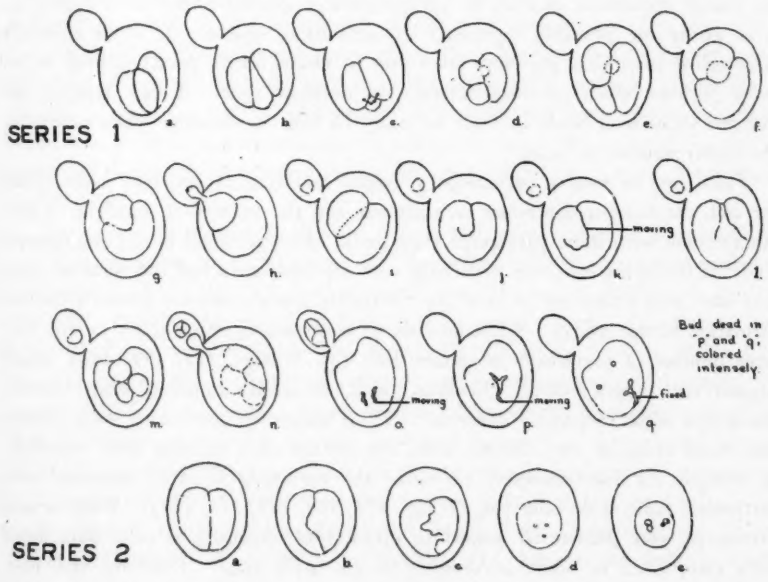
The cytoplasm of many living cells from presporulating cultures was so highly granular that all other structures were obscured. It was from these that spores developed. Although a number of methods of observing the formation of spores in living cells was tried, only the one described below was found satisfactory. Nothing externally visible occurred in the living cell the first twelve to sixteen hours on the gypsum slant, the variation in time depending upon the culture, temperature, presporulating medium, etc. After the culture had been on the gypsum slant for twelve hours samples were examined at half-hour intervals. The movements of yeast cells are sufficiently rapid that for accurate continued observation it was found imperative to restrain them as much as possible. The following technique prevented excessive motion, yet kept the yeast in viable condition under the microscope for five to seven hours. A tiny frayed-out fragment of lens paper was placed on a No. 1 cover glass and both were dipped in alcohol and flamed. With a little practice the lens paper fibers spread flat and evenly and are not charred. A drop of sterile water acidified to about pH 4 with acetic acid was placed on the lens paper and a few cells from the gypsum slant were added with a needle. The cover glass was placed cell-side down over a concave (drop) slide to provide an air chamber and the edges were sealed with wax. The

yeast cells gathered along the lens paper fibers, and Brownian movement was thus greatly reduced. Where the cells were not crowded, an observation every fifteen to twenty minutes kept a given cell or group of cells in view. Thus individual cells could be watched from the first sign of spore delimitation to complete spore formation, a process that took three to five hours at room temperature under these conditions. In acidified water with no air space, in agar, or in oil, sporulation also occurred but only sporadically and after one to five days.

In living cells the spores appeared to be delimited in the same manner as described for prepared slides. Several instances were observed in which the four spores were formed from the protoplasm at one end of a large cell. The other end was occupied by a body which resembled the magnicorps of other cells on the slide although no proof of its actual identity therewith was ascertained. A cell of this kind from a Giemsa-stained slide is illustrated in pl. 17, fig. 195. Generally the magnicorp was not visible in the living cells during the process of sporulation.

When iodine was applied to budding cells, it had the effect of temporarily bringing the parvicorp into "relief" and of emphasizing the particles in the magnicorp, often revealing them in rapid Brownian motion.

Toluidine blue was also applied to budding cultures. This dye at times caused



Text-fig. 3. Series of sketches of two cells under the influence of toluidine blue solution.

very striking reactions in the magnicorp, but the parvicorp was never visible. Changes in the magnicorp were especially apparent with continuous observation of a single cell. Text-fig. 3, Series 1, shows sketches of the changes in the magnicorp of a budding cell observed continuously for forty-five minutes. In "h" and "n" the magnicorp was seen to burst rapidly out into the bud. Series 2 shows a similar non-budding cell. Granules of various sizes often stain in the magnicorp with this procedure; occasionally also parts of the cytoplasm stain, and the cells finally die and become deeply colored throughout.

WILD YEASTS

Studies of Budding Cultures.—Several yeasts were collected in the wild and a few cytological observations were made on two of them. A brief account is given here of these fragmentary observations since they are rather suggestive when compared with the results with *Saccharomyces cerevisiae*. Had these yeasts been obtained earlier in the progress of the investigation more detailed observations on them would have been made.

The yeast represented in pl. 17 is one of a number which was isolated from wild fruit gathered late in the fall of 1945 at the Missouri Botanical Garden Arboretum near Gray Summit, Missouri. While it has not been positively identified, since it has not yet been induced to sporulate, it can be placed tentatively in the Fungi Imperfecti, possibly in *Syringospora* or *Blastodendrion* or a closely related genus and probably represents an undescribed species. It is an especially large-celled yeast that produces from two to twelve easily detached buds in bipolar position when growing actively on nutrient agar. A few Giemsa and Feulgen slides were made in order to study its cell organization when producing the higher number of buds.

From one to twelve parvicorps (Feulgen and Giemsa positive) were found per cell, the number depending on their size and the amount of budding. Commonly there were more parvicorps than buds. As was noted in the microscopic study of the living cells, the buds were very delicately attached and some of them may have been broken off in handling. Probably also the division precedes the bud formation in this species. While no attempt was made to check this fact, the very large number of parvicorps in certain cells (pl. 17, figs. 176, 177, 186) would suggest such a possibility. However, small, detached, immature buds without parvicorps were frequently observed. When numerous parvicorps were present they were often of two distinct sizes, the smaller ones staining more intensely. In contrast to *Saccharomyces cerevisiae* the parvicorp is often separated into particulate units at division (pl. 17, figs. 178, 180, 183, 187, 189). When several parvicorps were present all seemed to divide synchronously, as cells were noted with two, four, or eight in division at the same time. Dividing and nondividing parvicorps were not observed in the same cell. Wherever the magnicorp

was visible it was empty, but when many parvicorps were present they sometimes seemed to fill the whole cell.

Another of the wild yeasts from the same collection (a *Cryptococcus*?) should be mentioned because under certain cultural conditions (usually when several days to a week old) moving particles in the magnicorp were the rule rather than the exception and many of the particles seemed paired in dumbbell fashion. In some sections of one particular slide almost half the magnicorps had two equal particles, closely paired, each pair moving as a unit. Under other conditions, especially in young or older cultures, this phenomenon was not apparent.

Discussion

The very nature of vegetative reproduction in budding yeasts would lead one to expect their mitoses and nuclear organization to exhibit certain exceptional phenomena. As there is nothing quite comparable to this budding process in the plant or animal kingdom, interpretation of nuclear organization should take into account as inclusive a picture of all cell components as possible. Both parvicorp and magnicorp seem to be associated with vegetative cell division and a complete interpretation must take cognizance of both. From genetics there is now exact indirect evidence on the organization of the yeast cell. The excellent work of Lindegren, Spiegelman, and Lindegren ('44) and of Winge ('39, '44) has demonstrated the regular Mendelian segregation of characters at meiosis. Diploid strains, reproducing only vegetatively, are stable and retain their cultural characteristics as would be expected; haploid cultures exhibit a far greater mutation rate. This is easily observed in giant colony structures where haploids show frequent sector mutations which are generally lacking in the more stable diploids. Therefore in spite of its small size and peculiar vegetative reproduction yeast cannot be greatly different in its fundamental organization from other organisms although in various superficial details of mitosis and meiosis one may confidently expect considerable modification.

Similar superficial modifications of mitosis and meiosis have been reported for a variety of tissues and organisms. In the pollen tube of the spermatophytes, when mitosis of the generative cell must occur within narrow confines, it is often somewhat atypical; the metaphase plate may be greatly elongated and there may be no visible achromatic figure (Trankowski, '30). In the Protista, Belar ('26, '28) has shown that fundamentally regular but superficially atypical nuclear organization is not uncommon. As mentioned before, several conflicting interpretations of nuclear organization of yeast have been presented in the past and there is to date no general agreement. Table VI presents in tabular form the varying interpretations by previous authors of the disputed entities of the yeast cell.

In the light of the work of Mazia and Jaeger ('39), Caspersson and Schultz ('38), Caspersson ('39a, '39b, '40), Mirsky ('43), Mirsky and Pollister ('43a, '43b), Pollister and Mirsky ('43, '44), Greenstein ('44), Davidson and Way-

mouth ('44), and many others, the Feulgen positive staining of the parvicorp would seem indicative of its nuclear nature. The constancy of the parvicorp as a cell component and its non-homogeneous character when stained with Giemsa supports this interpretation. Opponents to this view point out that the division of the parvicorp appears to be amitotic. Since division is intra-nuclear in many fungi, this fact plus the very small size of the parvicorp make resolution of individual chromosomes difficult with the ordinary microscope. In the wild yeast described above particulate units were observed at certain division stages in both Feulgen and Giemsa slides. In the early stages of meiotic division in *S. cerevisiae* the parvicorp becomes enlarged and appears filamentous, resembling the usual early meiotic prophase nucleus, but its small size makes accurate resolution difficult. As is true of many nuclei, the parvicorp stains more intensely with Feulgen at some stages than it does at others. It is visible in all stages of division in all cells stained with Feulgen and Giemsa and with Heidenhain's hematoxylin and Brazilin if destaining is not carried too far, but is rarely visible in the living cells. In ultra-violet photographs (Caspersson and Brandt, '41; Brandt, '41) the two types of nucleic acid are indistinguishable. These authors state that this technique does not generally differentiate the nucleus in yeast. In the very actively budding cells, however, the parvicorps appear in the photographs as diffuse, somewhat lighter areas in the deeply absorbing cytoplasm, sometimes seeming not much more absorbent than the magnicorp which they state contains very little nucleic acid.

The magnicorp enters the bud before the parvicorp and is probably of importance in bud initiation as suggested by Lindegren ('45). If particles are visible within the magnicorp they also enter the bud, and at times they seem to be paired or assume form or position difficult to explain as "reserve stuff." These phenomena plus the apparent amitosis of the parvicorp have led to the interpretation (Janssens and Leblanc, '98; Wager and Peniston, '10; Lindegren, '45) of the magnicorp and its contents as the nuclear vacuole and chromatin respectively. Although the magnicorp and its contents are Feulgen negative at all stages of growth and division, Lindegren ('45) has pointed out that the desoxyribosenucleoprotein nature (Feulgen positive) of nuclei of higher organisms does not necessarily indicate its universality in the chromatin of simpler organisms. However, if the magnicorp is interpreted as the nuclear vacuole and the parvicorp as the centriole, the latter is not usually Feulgen positive. If visible, the particles in the magnicorp vary greatly as to size, shape, and number: (a) with physiological conditions—age, nutrition, aeration, etc.; (b) with the strain; and (c) with fixation. Their chemical composition is not known with certainty, but common nuclear fixing solutions are not generally effective, and special fixation, as, for example, with formaldehyde, must usually be employed. As pointed out earlier, the magnicorp is usually a single body, but often seems to occur as two bodies, one at either end of the parvicorp. In old or starved cultures it may appear to be divided into a number of parts (Brandt, '41), in which case the particles may occur in any or

all the parts of the magnicorp. Budding cells very commonly show no stained particles with the techniques employed. The number of particles counted in cells of different cultures stained with methylene blue-eosin varied from none in most cells to as high as fifty. Van Herwerden ('18) found the number of particles related to phosphorus content of the nutrient medium. With dark field illumination the rate of Brownian motion of particles in the magnicorp and in the surrounding medium is similar, indicating a very low viscosity. On the contrary, the lack of Brownian motion in the vicinity of the parvicorp and its tendency to bulge into the magnicorp would indicate a higher viscosity for the former. The pressure changes of the magnicorp with toluidine blue are likewise indicative of low viscosity, a character more usually associated with vacuolar behavior than with nuclear "sap." Difficulty in following any organization of the magnicorp through meiotic division in this investigation (also by Janssens and Leblanc, '98; and Wager and Peniston, '10) points strongly away from a nuclear interpretation but improved techniques may alter this. However, the presence of the magnicorp in the fully developed spore indicates its importance as a cell entity.

Various chromosome numbers in various parts of the cell have been suggested: two—both haploid and diploid—by Badian ('38); approximately eight—diploid —by Kater ('27) in the parvicorp; and twelve by Lindegren ('45) in the magnicorp. Although a number of investigators have noted the division of the parvicorp into two bodies at times and have called these chromosomes, present genetic evidence (Lindegren, personal communication, unpublished) does not point to this chromosome number, and cytological evidence is as yet uncertain. Undoubtedly the parvicorp is divided into two bodies at times, but if these are chromosomes they are large enough that they should be seen occasionally in metaphase or anaphase configuration. If the parvicorp is nuclear, further refinement of technique is necessary to be certain of chromosome number. If the magnicorp is nuclear, the variability of the number of particulate units is suspiciously great. However, because of the small size of the yeast cell and its lability under various cultural conditions, this objection has less force than it would have in other material. As mentioned previously, the parvicorp of the wild yeast described above appeared to separate into particulate units during mitosis but these were too crowded together for the number to be certain.

At least four parts of the yeast cell have been interpreted as the nucleolus (Table VI). Wager and Peniston ('10) considered the parvicorp as the nucleolus with the chromatin network extending from it over the magnicorp. This places the "nucleolus" entirely outside of the "nucleus" as they interpreted it, certainly an unusual position for it. Brandt ('41) and Caspersson and Brandt ('41) consider the ribonucleic acid-containing granules scattered throughout the cytoplasm (designated as "volutin" by them) the equivalent of the heterochromatin and nucleolus of higher organisms. Janssens and Leblanc ('98) sometimes called the central granule of the magnicorp the nucleolus, and Guilliermond ('20) and

TABLE VI

INTERPRETATIONS OF DISPUTED ENTITIES OF CELL ORGANIZATION BY VARIOUS INVESTIGATORS OF YEAST CYTOLOGY

Cell entity	Janssens and Leblanc, '98	Guilliermond, '03, '10, '20	Kohl, '08	Wager and Peniston, '10	Henneberg, '16	Kater, '27	Winge and Laustsen, '35, '37	Badian, '37	Beams et al., '39	Brandt, '41	Lindegren, '45	Subramaniam and Ranganathan, '45, '46a, '46b
Parvicorp	Part of nucleus (sometimes nucleolus?)	Nucleus containing nucleolus	Nucleus, possibly containing nucleolus	Nucleolus	Nucleus	Nucleus containing nucleolus (approx. 8 chromosomes)	Nucleus—probably 2 chromosomes	Nucleus—2 chromosomes in both haploid and diploid	Nucleus	Euchromatin of nucleus (2 chromosome equivalents)	Centriole (bipartite)	Nucleus—normally of 2 equal chromosomes
Magnicorp	Part of nucleus	Vacuole	Vacuole	Nuclear vacuole	Vacuole	Vacuole	Vacuole	Vacuole	Vacuole	Vacuole	Nuclear vacuole	——
Particles in magnicorp (may or may not be in motion)	?	Metachromatin (volutin)	Volutin	Chromatin network	Volutin	*No* particles in magnicorp	——	Volutin	——	Metachromatic granules	6 pairs of chromosomes	——

TABLE VI (continued)

Refractive, non-fatty cytoplasmic granules	?	Basophile granules	Volutin? and "Eiweiss-krystalle"	Volutin	Volutin?	Metachromatic granules (often a reticulum extending out into cytoplasm from parvicorp)	——	Volutin	——	Volutin—equivalent of heterochromatin and nucleolus of higher organisms also metachromatin	——	——
Companion body	?	——	——	Chromatin patch	——	——	——	——	——	——	Mentioned	——
Central granule of magnicorp (in motion)	Nucleolus?	——	——	Central volutin granule	——	——	——	——	——	——	Possibly "balled up" chromosomes	——

Kater ('27) thus designated the deeper-staining body or bodies in the parvicorp. At times one or more such bodies are visible in the Giemsa-stained slides, but whether these are sufficiently consistent to be considered the nucleoli is questionable.

Whether or not a membrane surrounds the parvicorp has been a question of debate. Its presence is indicated by the regularity of outline of the parvicorp after certain treatments, for example, with methylene blue-eosin and with Heidenhain's hematoxylin (short procedure; pl. 13, figs. 82–88; pl. 14, figs. 98–108). It is true that the parvicorps in Giemsa and Feulgen slides often show an irregular outline. Acid hydrolysis has been carried out with both of these, and although Bauer ('32) has demonstrated that this treatment does not alter the structure of the desoxyribosenucleoprotein-containing material, there is no reason to believe that a membrane, if present, would stain. Studies of the magnicorp indicate that there is a membrane separating it from the cytoplasm. At times it appears to be a double membrane for occasionally after fixation the magnicorp seems to shrink away from the cytoplasm in places as if both possessed membranes.

If the nucleo/cytoplasmic ratio is considered, the parvicorp alone gives a ratio which is possibly slightly low, the combined parvicorp and magnicorp an exceptionally high one.

It is thus apparent that after one hundred years of cytological work, the organization of the yeast nucleus is still a matter for debate among authorities, even as to the most elementary points. Direct observation, uncorrelated with other techniques, must probably continue to be relatively unproductive with objects as microscopically refractory as the yeast cell. However, direct cytological observation in combination with other techniques presents a more encouraging picture. Among the most promising of these developments are the following:

(1) Additional genetic studies such as those of Lindegren and Winge. Eventually they should demonstrate the chromosome numbers, their comparative size, and even chiasma frequencies and positions.

(2) Further biochemical studies along the lines of those of Mirsky and Pollister on higher organisms, combined with studies of the effects of enzymatic action on the several cell entities. A more complete knowledge of the chemical nature of the particles in the magnicorp and of the refractive granules in the cytoplasm should aid in an understanding of their role in cell organization and metabolism.

(3) Comparative cytological studies of wild yeasts. Species with larger cells or clearer cytoplasm should reveal more details of cell structure. Wild yeasts which reproduce by budding but which also develop true or pseudo-mycelia are frequently encountered. A cytological study of these species would relate the recognized entities of the yeast cell to the more usual mycelial type of growth.

(4) Additional investigations with ultra-violet and "phase-difference" microscopes and the study of thin sections with the electron microscope.

(5) Further refinements of cytological techniques. A method of removing, softening, or breaking the firm cell wall (possibly by enzymatic digestion, chem-

ical maceration, or pressure) which would permit spreading of the cell contents without excessive distortion should make further details of the parvi- and magnicorp resolvable. A study of the effects of cold treatment on different stages of spore formation may make it possible to obtain many more cells at one time in certain phases of meiosis. New techniques, as well as improvements in present fixation and staining procedures, should provide additional information for correlation with other methods.

Summary

1. A review of the literature on the cytology of yeasts showed great confusion in:

(1) use of terms,

(2) interpretation of nuclear structure.

(This is demonstrated in tabular form in Table VI.) An attempt was made to apply systematically a whole battery of techniques to both sporulating and budding material of yeast *(S. cerevisiae)*.

2. All of the techniques tried out are listed and described. Those used extensively are discussed in detail.

3. A descriptive terminology for the major cell entities (parvicorp, magnicorp, companion body, text-fig. 1, p. 254) is adopted in order to permit presentation of observations unbiased by earlier interpretations.

4. Fragmentary observations on two wild yeasts are included for comparison (pp. 264–265).

5. The "parvicorp" is a Feulgen positive, non-homogeneous, constant cell entity. Vegetative division does not appear to be typically mitotic in *S. cerevisiae*, but at least the prophase of meiosis resembles conventional configurations. A large-celled wild yeast showed particulate units of the parvicorp at mitosis.

6. The "magnicorp" (vacuole or nuclear vacuole of most authors) is Feulgen negative. It is almost universally present in budding material and enters the bud before the parvicorp, but is generally invisible in presporulating and sporulating material. Granules of the magnicorp are lacking in most cells with the techniques employed, but, when present, occasionally appear paired.

7. Relationships of these observations to the several interpretations of nuclear organization are discussed briefly (pp. 265–271), and suggestions are made as to possible future advances in this field.

LITERATURE CITED

Badian, J. (1937). Sur la cytologie des Levures. Acad. Polon., Bull. B, 1:61–87.

Bauer, H. (1932). Die Feulgensche Nuclealfarbung in ihrer Anwendung auf cytologische Untersuchung. Zeitschr. Zellforsch. 15:225–247.

Beams, H. W., L. W. Zell, and N. M. Sulkin (1940). Cytological studies on yeast cells with special reference to the budding process. Cytologia 11:30–36.

Belar, K. (1926). Zur Cytologie von *Aggregata eberthi*. Arch. Prot. 53:312–325.

———, (1928). Die cytologischen Grundlagen der Vererbung. Berlin.

Belozerskii, A. N. (1945). The chemical nature of volutin. Microbiology (USSR) 14:29–34. [Chem. Abs. 40:616–617.]

Bensley, R. R., and S. H. Bensley (1938). Handbook of histological and cytological technique. Chicago.

Brandt, K. M. (1941). Physiologische Chemie und Cytologie der Presshefe. Protoplasma 36:77–119.

Caspersson, T. (1939a). On the role of the nucleic acids in the cell. Proc. 7th Int. Genet. Cong. 85–86.

———, (1939b). Über die Rolle der Desoxyribosenukleinsäure bei der Zellteilung. Chromosoma 1:147–156.

———, (1940). Nucleinsäureketten und Genvermehrung. *Ibid.* 1:605–619.

———, und K. M. Brandt (1941). Nucleotidumsatz und Wachstum bei Presshefe. Protoplasma 35:507–526.

———, and J. Schultz (1938). Nucleic acid metabolism of the chromosomes in relation to gene reproduction. Nature 142:294–295.

Coleman, L. C. (1938). Preparation of Leuco basic fuchsin for use in the Feulgen reaction. Stain Technol. 13:123–124.

Dangeard, M. P. A. (1916). Note sur les corpuscules métachromatiques des Levures. Soc. Myc. de France, Bull. 32:27–32.

Darlington, C. D., and L. F. LaCour (1942). The handling of chromosomes. New York.

Davidson, J. N., and C. Waymouth (1944). The histochemical demonstration of ribonucleic acid in mammalian livers. Roy. Soc. Edinburgh, Proc. 62:96–98.

de Tomasi, J. A. (1936). Improving the technique of the Feulgen stain. Stain Technol. 11:137–144.

Dubos, R. J. (1945). The bacterial cell. (with Addendum by C. F. Robinow). Cambridge, Mass.

Greenstein, J. P. (1944). Nucleoproteins. Adv. in Protein Chem. 1:210–287.

Guilliermond, A. (1903). Recherches cytologiques sur les Levures. Rev. Gén. Bot. 15:49–66.

———, (1910). Nouvelles observations sur la cytologie des Levures. Compt. Rend. Acad. Sci., Paris 150:835–838.

———, (1920). The yeasts. Translated by F. W. Tanner. New York.

Hancock, B. L. (1942). A schedule for chromosome counts in some plants with small chromosomes. Stain Technol. 17:79–83.

Henneberg, W. (1916). Über das Volutin (= metachromatische Körperchen) in der Hefezelle. Centralbl. f. Bakt. II, 45:50–62.

Hillary, B. B. (1939). Use of the Feulgen reaction in cytology. I. Effect of fixatives on the reaction. Bot. Gaz. 101:276–300.

Janssens, F. A., et A. Leblanc (1898). Recherches cytologiques sur la cellule de Levure. La Cellule 14:201–243.

Johansen, D. A. (1940). Plant microtechnique. New York.

Kater, J. McA. (1927). Cytology of *Saccharomyces cerevisiae* with especial reference to the nuclear division. Biol. Bull. 52:436–446.

Kohl, F. G. (1908). Die Hefepilze. Leipzig.

LaCour, L. (1941). Acetic orcein: a new stain-fixative for chromosomes. Stain Technol. 16:169–174.

Lee, A. B. (1928). The microtomist's vade-mecum, 9th ed. Edited by Gatenby, J. and E. V. Cowdry. Philadelphia.

———, (1937). The microtomist's vade-mecum, 10th ed. Edited by Gatenby, J. and E. V. Cowdry. Philadelphia.

Lindegren, C. C. (1945). An analysis of the mechanism of budding in yeasts and some observations on the structure of the yeast cell. Mycologia 37:767–780.

———, and Gertrude Lindegren (1943). Environmental and genetical variations in yield and colony size of commercial yeasts. Ann. Mo. Bot. Gard. 30:71–83.

———, ———, (1944). Sporulation in *Saccharomyces cerevisiae*. Bot. Gaz. 105:304–316.

———, S. Spiegelman, and Gertrude Lindegren (1944). Mendelian inheritance of adaptive enzymes in yeast. Nat. Acad. Sci., Proc. 30:346–352.

Margolena, L. A. (1932). Feulgen's reaction and some of its applications for botanical material. Stain Technol. 7:9–16.

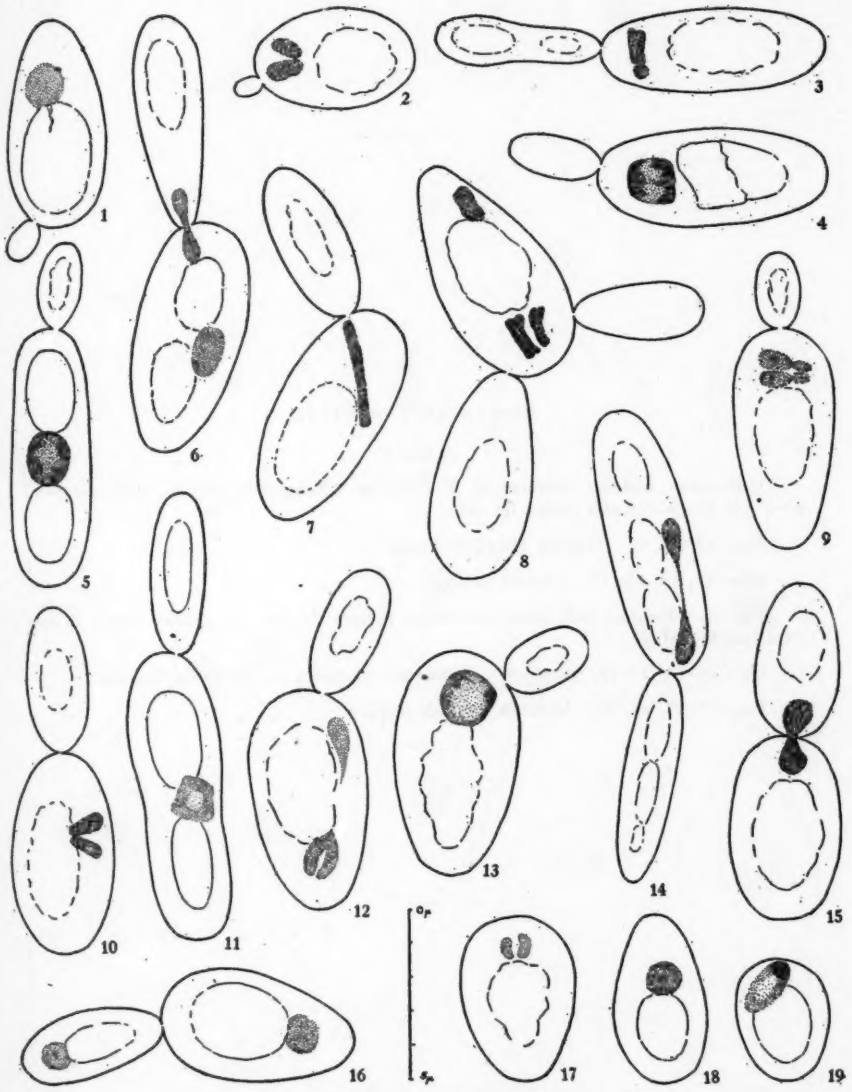
Mazia, D., and L. Jaeger (1939). Nuclease action, protease action and histochemical tests on salivary chromosomes of *Drosophila*. Nat. Acad. Sci., Proc. **25**:456–461.
Meyer, A. (1904). Orientirende Untersuchung über Verbreitung, Morphologie und Chemie des Volutins. Bot. Zeitung **62**:113–153.
Mirsky, A. E. (1943). Chromosomes and nucleoproteins. Adv. in Enzymology **3**:1–35.
———, and A. W. Pollister (1943a). Studies on the chemistry of chromatin. N. Y. Acad. Sci., Trans. II, **5**:190–198.
———, ———, (1943b). Fibrous nucleoproteins of chromatin. Biol. Symposia **10**:247–260.
Moore, Morris (1933). A study of *Endomyces capsulatus* Rewbridge, Dodge and Ayers: a causative agent of fatal cerebrospinal meningitis. Ann. Mo. Bot. Gard. **20**:471–548.
Nägeli, C. (1844). On the nuclei, formation and growth of vegetable cells. Ray Society **1845**:213–292. (Translated from Schleiden u. Nägeli's Zeitschr. f. Wiss. Bot. 1844 by Henfrey.)
Pollister, A. W., and A. E. Mirsky (1943). Terminology of nucleic acids. Nature **152**:692–693.
———, ———, (1944). Distribution of nucleic acids. Nature **152**:711.
Robinow, C. F. (1942). A study of the nuclear apparatus of bacteria. Roy. Soc. London, Proc. B. **130**:299–324.
Semmens, C. S. (1942). Improved cytological methods with crystal violet. Stain Technol. **17**:147–148.
Smith, F. H. (1934). The use of picric acid with the gram stain in plant cytology. Stain Technol. **9**:95–96.
Subramaniam, M. K., and B. Ranganathan (1945). Mitosis during budding in *Saccharomyces cerevisiae*. Current Sci. **14**:78–79.
———, ———, (1946a). A new mutant of *Saccharomyces cerevisiae*. Nature **157**:49–50.
———, ———, (1946b). Peculiar cytological behaviour of a distillery yeast. Nature **157**:50–51.
Thomas, P. T. (1940). The aceto-carmine method for fruit material. Stain Technol. **15**:167–172.
Trankowsky, D. A. (1930). Zytologische Beobachtungen über die Entwicklung der Pollenschläuche einiger Angiospermen. Planta **12**:1–18.
Tuan, H. (1930). Picric acid as a destaining agent for iron alum. Stain Technol. **5**:135–138.
van Herwerden, M. A. (1918). On the nature and significance of volutin in yeast-cells. Akad. Amsterdam, Proc. **20**[1]:70–87.
Wager, H. (1898). The nucleus of the yeast plant. Ann. Bot. **12**:499–543.
———, and Annie Peniston (1910). Cytological observations on the yeast plant. *Ibid.* **23**:45–84.
Warmke, H. E. (1935). A permanent root-tip smear method. Stain Technol. **10**:101–103.
Winge, O. (1935). On haplophase and diplophase in some Saccharomyces. Lab. Carlsberg, Compt. Rend. Ser. Physiol. **21**:77–118.
———, (1944). On segregation and mutation in yeast. *Ibid.* **24**:79–95.
———, and O. Laustsen (1937). On two types of spore germination, and on genetic segregations in *Saccharomyces*, demonstrated through single-spore cultures. *Ibid.* **22**:99–120.
———, ———, (1939). *Saccharomycodes Ludwigii* Hansen, a balanced heterozygote. *Ibid.* **22**:357–374.
Zikes, H. (1922). Beitrag zum Volutinvorkommen in Pilzen. Centralbl. f. Bakt. II, **57**:21–45.

Explanation of Plate

PLATE 10

Cells from budding culture of *Saccharomyces cerevisiae* (Feulgen technique).

Figs. 1–19. Shaken broth culture, diploid (LK) strain, mercuric chloride fixation.



NAGEL—SACCHAROMYCES CEREVISIAE

Explanation of Plate

PLATE 11

Cells from budding cultures of *S. cerevisiae* (Robinow's Giemsa technique)—all 12–24-hr. broth cultures except fig. 46.

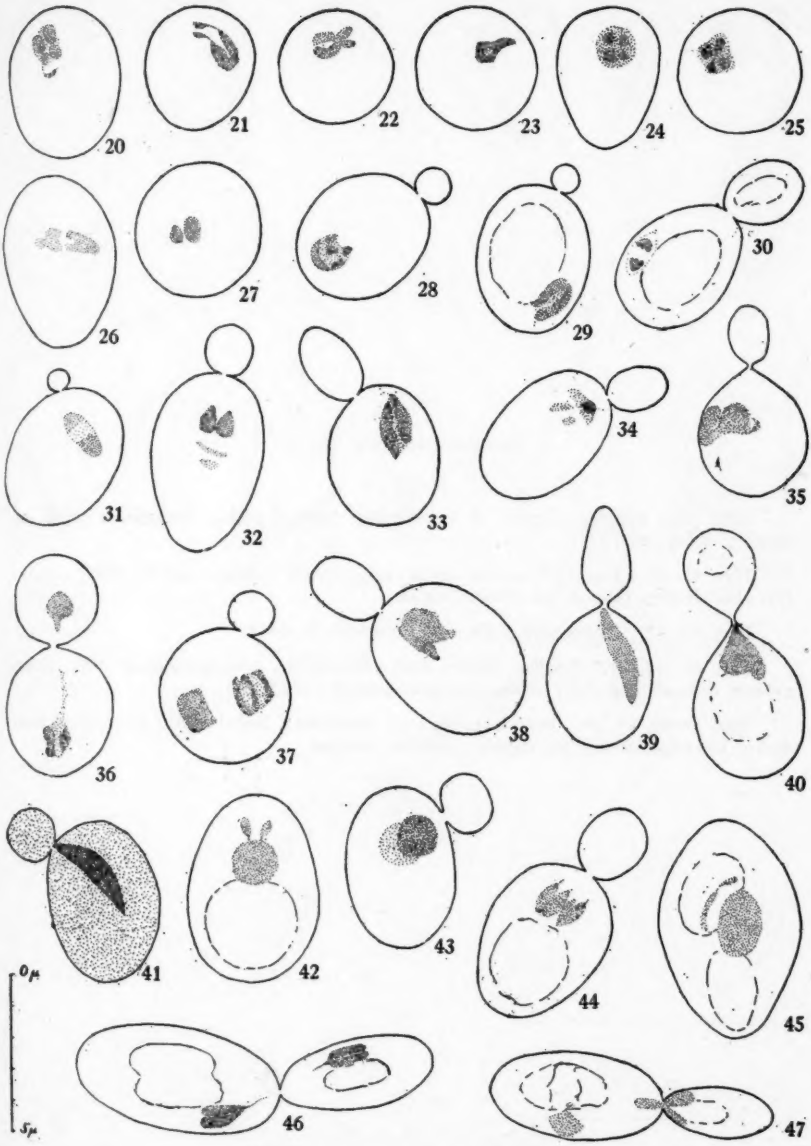
Figs. 20–41, 43. Haploid (Bc20—) strain.

Figs. 42, 44, 45, 47. Diploid strains.

Fig. 46. Budding cell from sporulating culture 24 hrs. on gypsum slant, diploid intra-specific hybrid.

Figs. 20–34, 38–42, 44, 45, 47. Iodine-glacial acetic acid-formalin fixation.

Figs. 35–37, 43, 46. Mercuric chloride fixation.



Budding material – Giemsa

NAGEL—SACCHAROMYCES CEREVISIAE

Explanation of Plate

PLATE 12

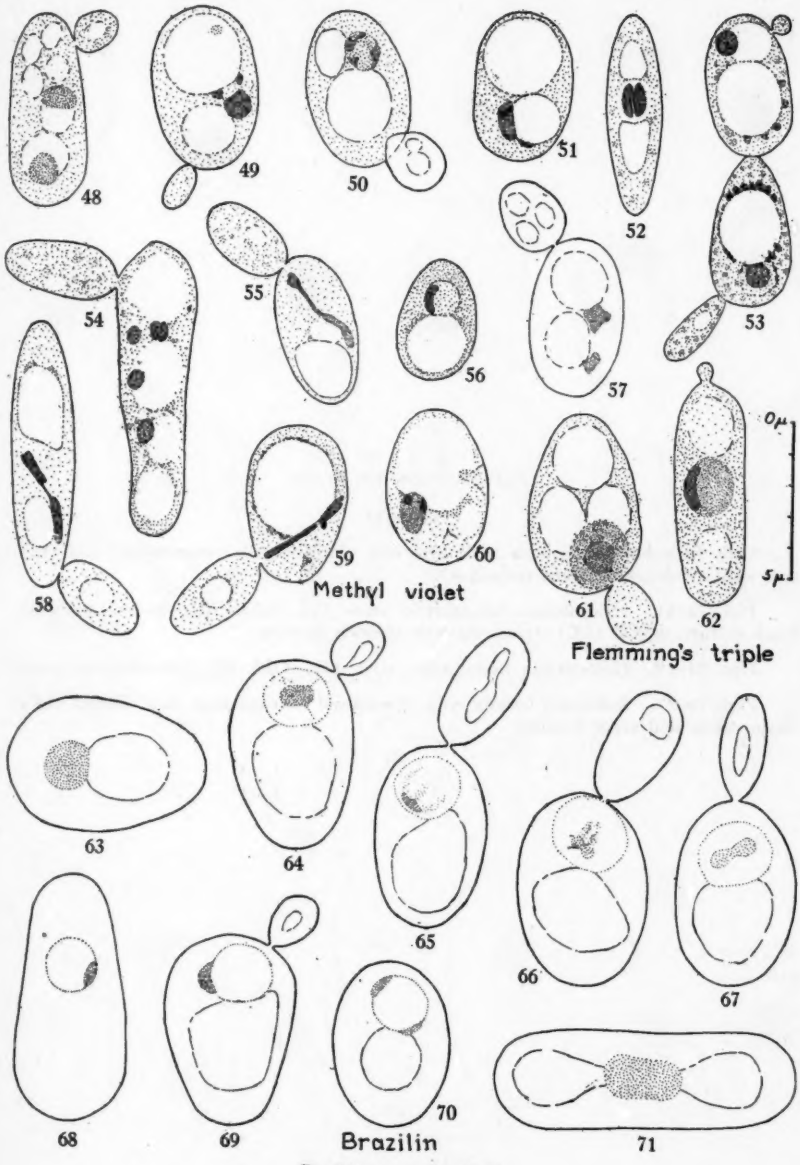
Cells from budding cultures of *S. cerevisiae* (methyl violet, Flemming's triple, or Brazilin technique).

Figs. 48–60. Johansen's methyl violet stain, broth culture, diploid (LK) strain, Navashin fixation (figures not drawn to scale).

Figs. 61, 62. Flemming's triple stain, otherwise as above.

Figs. 63, 68, 71. Brazilin (with short destaining), non-sporulating cells from gypsum slant, diploid (LK) strain, mercuric chloride fixation.

Figs. 64–67, 69, 70. Brazilin (with long destaining), broth culture from yield test, diploid intra-specific hybrid, mercuric chloride fixation.



NAGEL—SACCHAROMYCES CEREVISIAE

Explanation of Plate

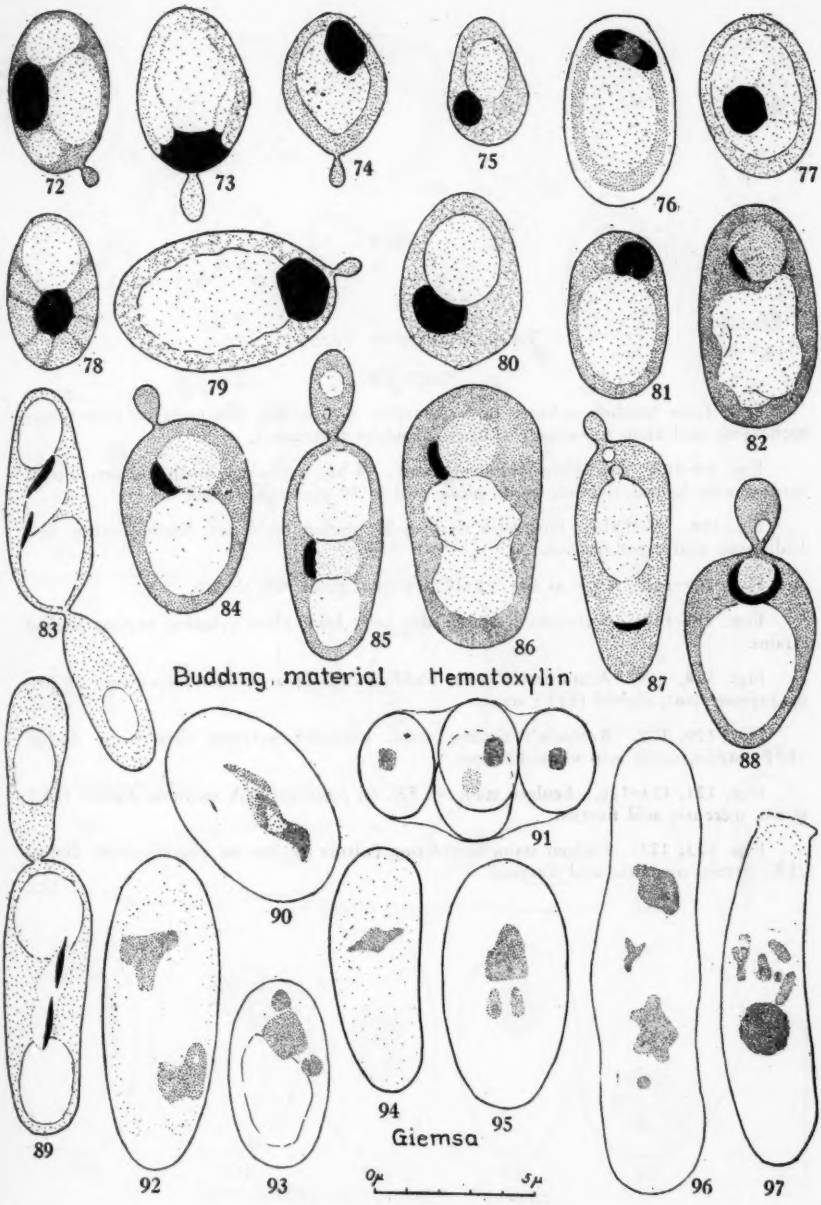
PLATE 13

Cells from budding culture of *S. cerevisiae* (Heidenhain's hematoxylin) and from agar slant (Robinow's Giemsa technique).

Figs. 72–81. Heidenhain's hematoxylin stain (5a, Table III), 24-hr., unshaken broth culture, diploid (LK) strain, mercuric chloride fixation.

Figs. 82–89. Heidenhain's hematoxylin stain (5b, Table III), otherwise as above.

Figs. 90–97. Robinow's Giemsa stain, 7-week-old nutrient agar slant, diploid (LK) strain, osmic acid vapor fixation.



NAGEL—SACCHAROMYCES CEREVISIAE

Explanation of Plate

PLATE 14

Cells from budding cultures of S. *cerevisiae* (methylene blue-eosin or aceto-orcein technique) and from sporulating cultures (Feulgen technique).

Figs. 98–107. Methylene blue-eosin stain, 48-hr., unshaken broth culture, diploid intra-specific hybrid, fixation in sat. picric acid in 70 per cent alcohol.

Fig. 108. Methylene blue-eosin stain; 4-hr. shaken yield test, broth culture, diploid, osmic acid vapor fixation.

Figs. 109–112. Same as figs. 98–107 except culture was shaken.

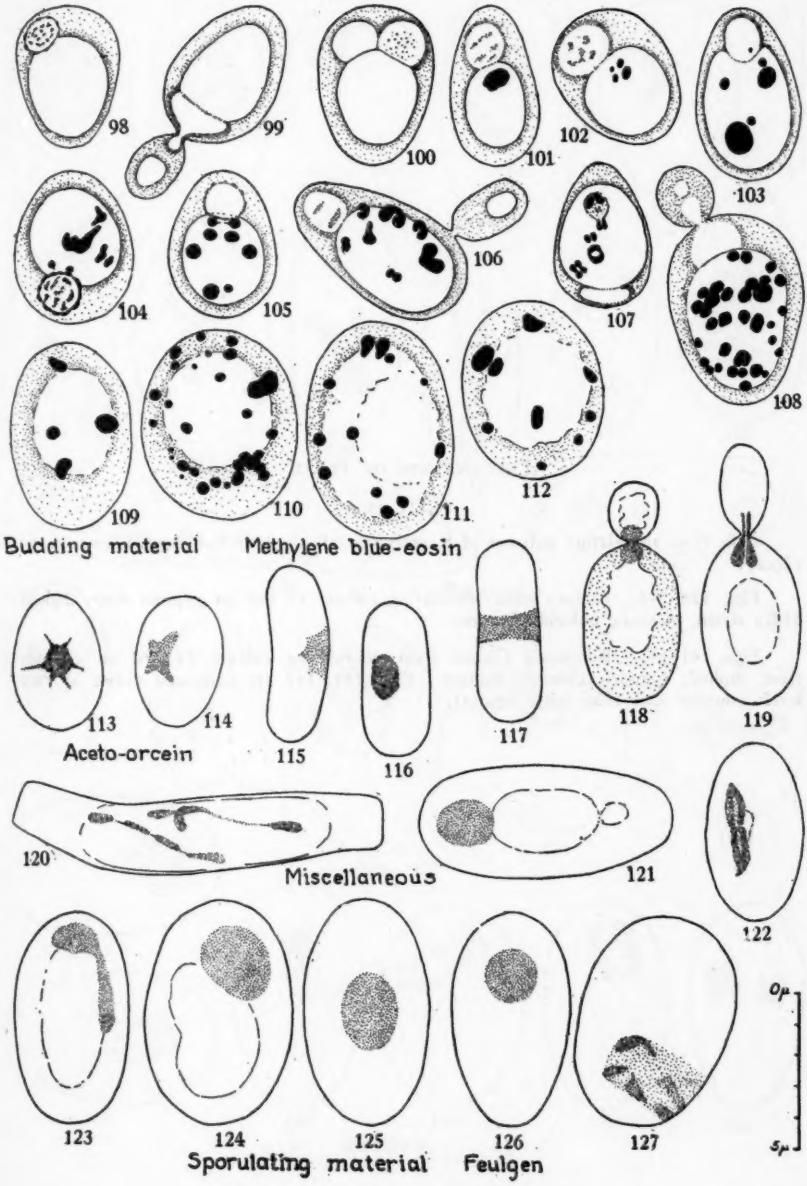
Figs. 113–117. Aceto-orcein stain, edge cells from giant colonies, various haploid strains.

Figs. 118, 119. Aceto-orcein stain, budding cells from sporulating culture 24 hrs. on gypsum slant, diploid (EFF) strain.

Figs. 120, 122. Robinow's Giemsa stain, week-old nutrient agar slant, diploid (EFF) strain, osmic acid vapor fixation.

Figs. 121, 124–126. Feulgen stain, 48 hrs. on presporulating medium, diploid (LK) strain, mercuric acid fixation.

Figs. 123, 127. Feulgen stain, sporulating culture 17 hrs. on gypsum slant, diploid (LK) strain, mercuric acid fixation.



NAGEL—SACCHAROMYCES CEREVISIAE

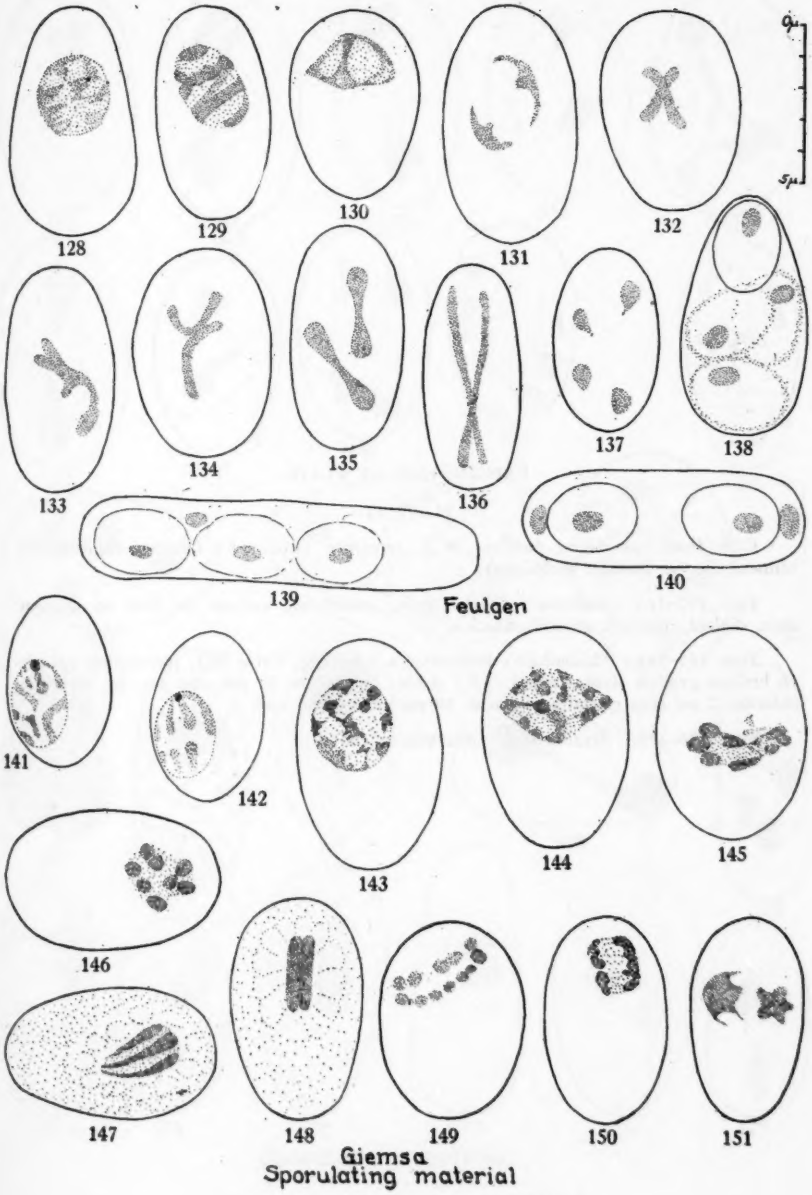
Explanation of Plate

PLATE 15

Cells from sporulating cultures of *S. cerevisiae* (Feulgen or Robinow's Giemsa technique).

Figs. 128–140. Feulgen stain, sporulating culture 17 hrs. on gypsum slant, diploid (LK) strain, mercuric chloride fixation.

Figs. 141–151. Robinow's Giemsa stain, sporulating culture 24 hrs. on gypsum slant, diploid, mercuric chloride fixation. Figs. 141, 142 are same cell shown at two levels (smaller scale than other figures).



NAGEL—SACCHAROMYCES CEREVISIAE

Explanation of Plate

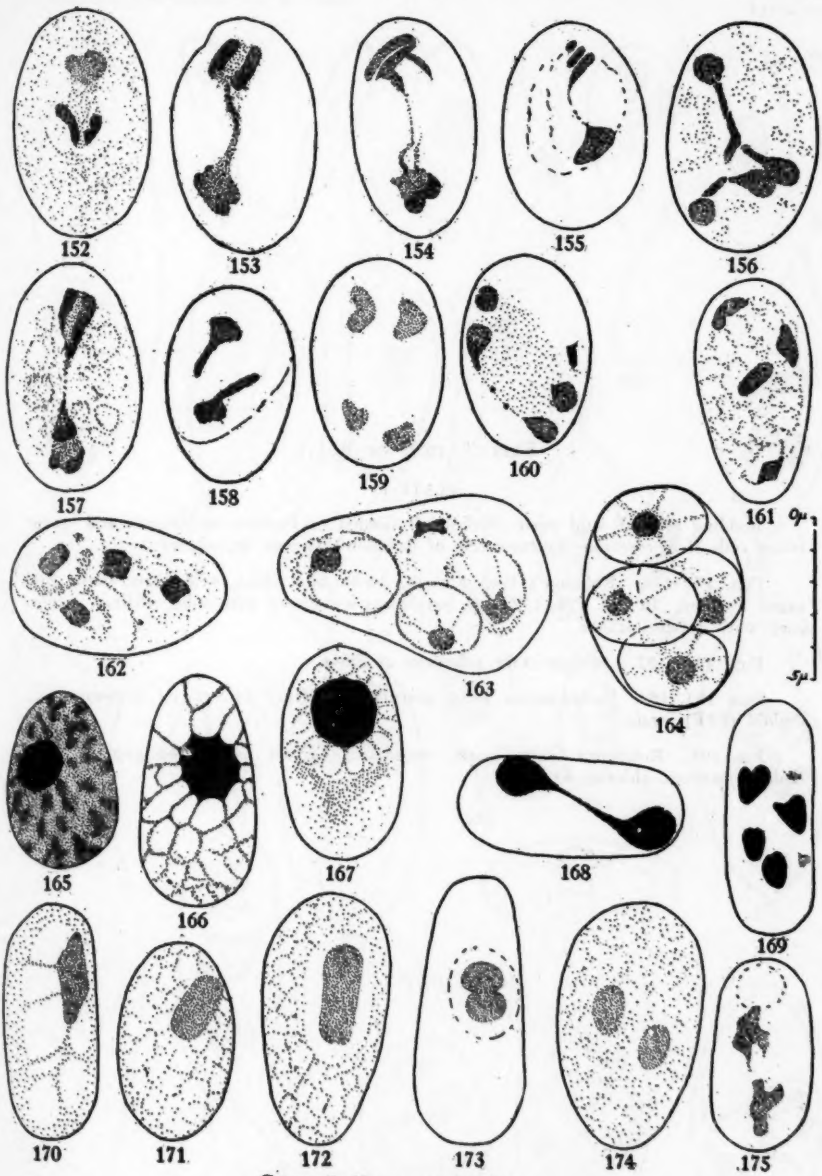
PLATE 16

Cells from sporulating cultures of *S. cerevisiae* (Robinow's Giemsa, Heidenhain's hematoxylin, or Brazilin technique).

Figs. 152–164. Robinow's Giemsa stain, sporulating culture 24 hrs. on gypsum slant, diploid, mercuric chloride fixation.

Figs. 165–169. Heidenhain's hematoxylin stain (5a, Table III), sporulating culture 16 hrs. on gypsum slant, diploid (LK) strain, fixation in 48 per cent sat. aq. mercuric chloride, 2 per cent glacial acetic acid, 50 per cent picric acid.

Figs. 170–175. Brazilin stain, otherwise as above.



NAGEL—SACCHAROMYCES CEREVISIAE

Explanation of Plate

PLATE 17

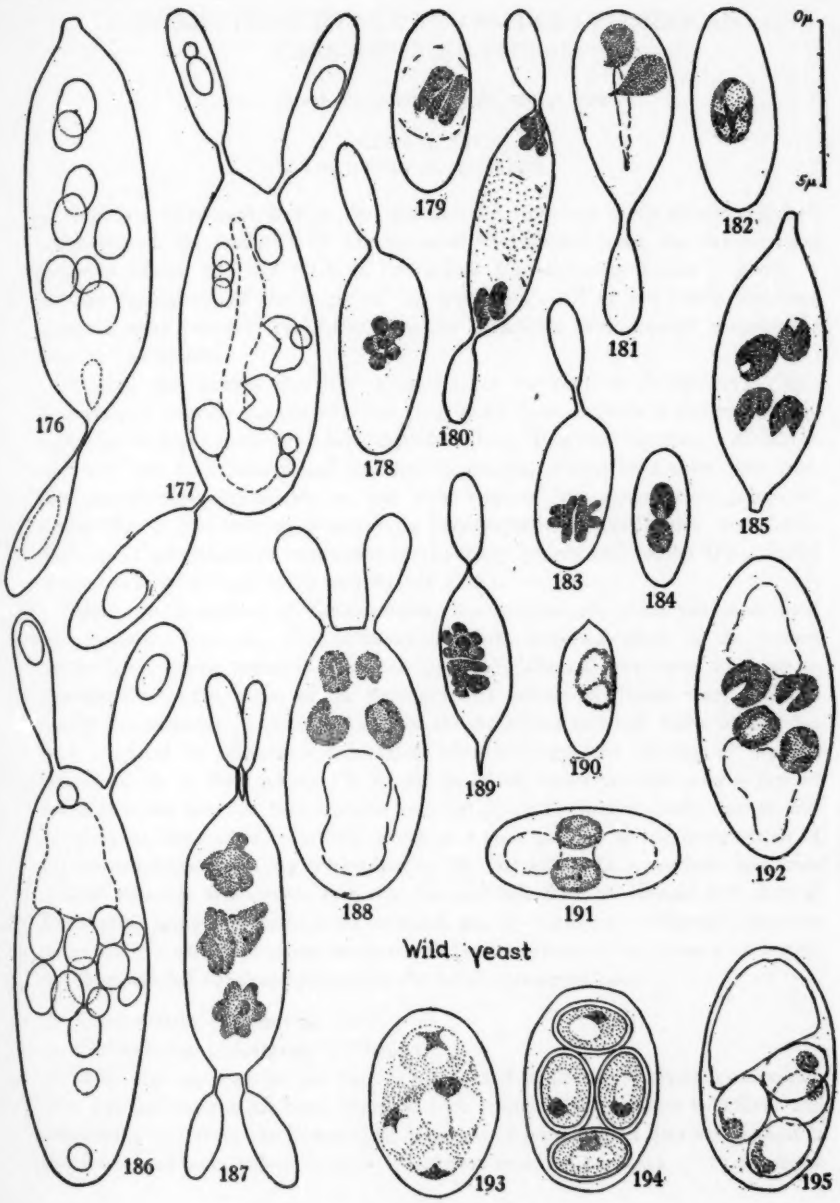
Budding cells of wild yeast (Robinow's Giemsa or Feulgen technique) and sporulating cells of S. *cerevisiae* (aceto-orcein or Robinow's Giemsa technique).

Figs. 176–190. Robinow's Giemsa stain, 24-hr. agar slant, wild yeast, osmic acid vapor fixation. In figs. 176, 177, 186 parvicorps are shown with solid outlines, magnicorps with dotted outlines.

Figs. 191, 192. Feulgen stain, otherwise as above.

Figs. 193, 194. Aceto-orcein stain, sporulating culture 24 hrs. on gypsum slant, diploid (EFF) strain.

Fig. 195. Robinow's Giemsa stain, sporulating culture 24 hrs. on gypsum slant, diploid, mercuric chloride fixation.



NAGEL—SACCHAROMYCES CEREVISIAE

CONTRIBUTIONS TO OUR KNOWLEDGE OF AMERICAN CARBONIFEROUS FLORAS

IX. Some Petrified Seeds from Iowa

ELLEN M. KERN
AND HENRY N. ANDREWS

This is a third contribution that has accrued from our study of the coal-ball collections of Mr. Frederick O. Thompson of Des Moines, Iowa, the two previous accounts (Parts VII and VIII of "American Carboniferous Floras") giving a detailed description of the origin of the specimens. All of the fossils described herewith were found in coal balls from the Urbandale mine located immediately west of Des Moines.

During the decades that have elapsed since the time of Brongniart's ('81) foundational memoir on the silicified seeds from Saint-Etienne a rather vast assemblage of fossil seeds have been described from Paleozoic deposits. Although relatively few have been found attached to the plants that bore them they have contributed very appreciably to our knowledge of the three great groups of Carboniferous seed-bearing plants—the Pteridospermeae, Cordaitales, and Lycopodiales. Comprehensive summaries of the many genera and species are included in the works of Seward ('17) and Arnold ('38).

With the exception of *Lepidocarpon*, few structurally preserved seeds have been recorded from the Carboniferous of North America, most of the known species having been found in European deposits. There is now some evidence to indicate that certain areas of the Pennsylvanian forests of Illinois were predominantly pteridophytic, and since much of the American coal-ball work to date has been confined to collections from that state the apparent paucity of seeds is accounted for at least in part. It should be added, however, that only a few of the coal mines of even Illinois have been subjected to intense study, and it will certainly be some years before we arrive at a clear picture of the composition of the ancient forests that are represented in the coal balls. In a previous discussion a brief contrast was drawn between the coal-ball flora of Illinois and that of Iowa, seeds being present in Iowa in much greater numbers. Although there are at least seven species of seeds, exclusive of *Lepidocarpon*, in our present collection we have selected for description only the better-preserved ones.

Conostoma Williamson, 1877.

Conostoma oblongum Williamson.

Only one specimen of this species has turned up in our Urbandale collection thus far, and since it has been described with considerable precision by Oliver and Salisbury ('11) from the Lower Coal Measures of Lancashire a detailed account is not warranted here. More recently it has been reported by Krick ('32) from the

upper part of the Carbondale group at Harrisburg, Illinois.

The Iowa record given here is based on a specimen that was exposed in longitudinal section, although the cut had been made slightly beyond the median plane. The seed (pl. 18, fig. 2) measures nearly 4.5 mm. long by 2 mm. in diameter and shows no pronounced tapering toward either end. The apical portion of the nucellus (fig. 3) is quite well preserved, showing the distinctive generic features of the pollen chamber in which two pollen grains may be noted. The integument is lobed at the micropylar end, somewhat more distinctly so than in the previously described specimens.

Conostoma oblongum is described by Oliver and Salisbury as being platyspermic although in their own words it is "only trifling in amount," and their figures adequately bear this out. In view of the general wide range in cross-sectional shape of the petrified Carboniferous seeds there can be little doubt that more fundamental structural features, such as the organization of the pollen chamber and the nature of the integument, are of greater significance. In other words, Seward's classification of the Paleozoic seeds into three groups, the Lagenostomales, Trigonocarpales, and Cardiocarpales, is certainly preferable to the older system based on cross-sectional shape.

Aside from Krick's citation of *Conostoma oblongum* in an Illinois coal ball, the only previously known American species are those described by Graham ('34). His well-executed figures of *C. platyspermum* leave no doubt as to the generic identity of this fossil, and although it is very close to *C. oblongum* its segregation as a distinct species seems justified.

Rhabdospermum Seward, 1917.

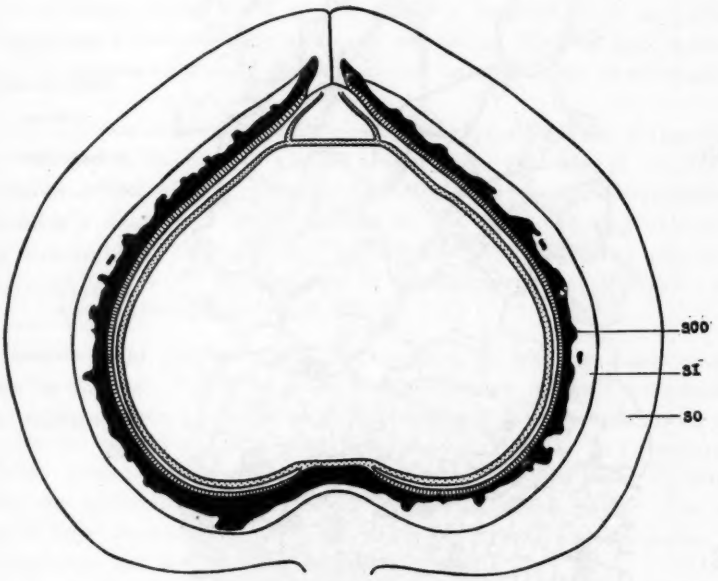
Rhabdospermum spinatum Andrews, sp. nov.

The fossil seeds, preserved as both petrifactions and impressions, that are of Cordaitean affinities already compose a striking assemblage displaying considerable variation in size, form, and anatomy. The fact that the vast majority of these seeds (included in the Cardiocarpales as classified by Seward, '17, pp. 332-356) have been described from European Carboniferous deposits by no means implies their restriction to that region. Cordaitean stem, root, and foliage remains are of frequent occurrence in the Illinois and Iowa coal balls. Although the present description is based on a single, incomplete seed, it seems worth recording inasmuch as it presents characters that distinguish it from any previously described species.

The seed is heart-shaped in longitudinal section and relatively large, measuring 15 mm. broad by 12 mm. long. The integument, which is composed of four clearly defined tissues, is especially well preserved. Following the nomenclature used by most previous workers, the outer two tissues will be referred to as the *sarcotesta* and the inner, more sclerotic two, as the *sclerotesta* (text-figs. 1, 2).

The outer sarcotesta, which is appreciably thicker than the three inner zones

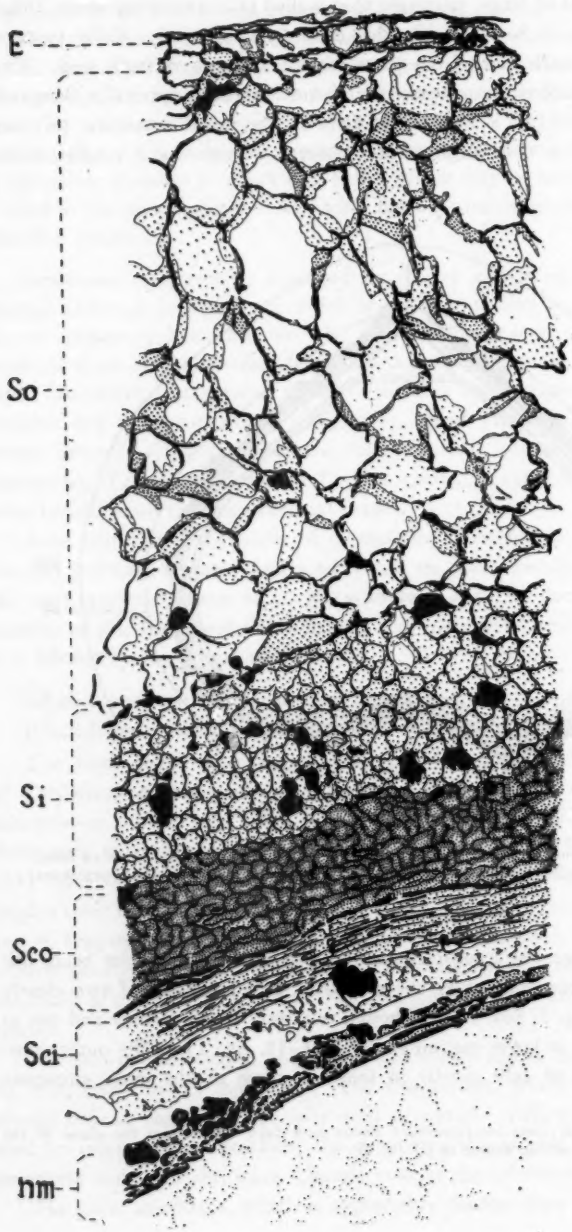
combined, is composed of large, relatively thin-walled cells averaging about 200μ[1] in diameter (text-fig. 2, So). In life this must have been of a fleshy texture, probably comparable with that of the outer coat of a *Cycas circinalis* seed. This outer sarcotesta is bounded by an epidermis of much smaller, vertically elongated cells averaging about 115μ x 70μ. The cells of the inner sarcotesta (Si) are nearly isodiametric, like those of the outer tissue, although much smaller, averaging 50μ.



Text-fig. 1. *Rhabdospermum spinatum.* Diagram of the seed shown in median longitudinal section, with pollen chamber restored: SO, outer sarcotesta; SI, inner sarcotesta; SCO, outer sclerotesta.

The sclerotesta appears as a conspicuous dark brown band, the color being due partly to the cellular contents. Like the sarcotesta, it is composed of two clearly defined tissues (text-fig. 2, Sco, Sci), although relatively much thinner and not as readily distinguishable at lower magnifications (pl. 18, fig. 1). The outer component (Sco) consists of cells similar in shape to those of the inner sarcotesta

[1]All cell measurements of these integumentary tissues have been taken from the plane of the nearly median longitudinal section shown in pl. 18, fig. 1.



Text-fig. 2

Rhabdospermum spinatum

Cellular detail taken from the equatorial region: E, epidermis; So, outer sarcotesta; Si, inner sarcotesta; Sco, outer sclerotesta; Sci, inner sclerotesta; nm, remnants of nucellus and megaspore membrane.

although thicker-walled. The most conspicuous feature of this tissue is the irregularly shaped spines projecting into the inner sarcotesta, and it is in recognition of this character that the specific name has been chosen. The inner sclerotesta consists of cells that are very poorly preserved but which apparently were elongated in the plane of the longitudinal axis.

The remnants of the nucellus are clearly attached to a raised cushion at the base of the seed although it otherwise appears to be quite free from the integument. It should be noted that, since the innermost tissues of the sclerotesta and of the adjoining nucellar tissue are not perfectly preserved, it is not certain whether the two were originally in organic connection. Yet the space between them is so uniform as to imply that it is natural rather than due to shrinkage or decay.

Very little remains of the nucellar tissue. At its apex, however, it appears to have developed in the form of a broad conical pollen chamber (pl. 18, fig. 1, text-fig. 1). Although little more than the cuticularized remains of the epidermal cells are left, the form of the integument in this region also suggests such a terminal structure of the nucellus. Thus the internal conical cavity presents a distinct contrast to the flattened one in *Rhabdospermum cyclocaryon* (Ad. Brongn.) Seward (Brongniart, '81, pl. XII, fig. 1).

Affinities:—The affinities of this seed appear to lie with the closely related genera *Cardiocarpus, Rhabdospermum* and *Mitrospermum* of the Cardiocarpales. The last two of these genera are distinguished from *Cardiocarpus* chiefly in the way that the vascular strands originate from the main bundle. In *Cardiocarpus* the outer (lower) bundles depart from the main vascular axis before the latter reaches the sclerotesta, while in *Rhabdospermum* and *Mitrospermum* they are given off from the sclerotesta region and recurve back into the outer portion of the integument (cf. Seward, '17, figs. 500B and 501E).

As stated above, the only available specimen of this seed was exposed on a previously cut slab from Mr. Thompson's collection. The fact that the initial cut was apparently made directly through the central vascular strand leading up through the base of the seed makes it impossible to determine whether the integument branches departed from the main bundle before or after reaching the sclerotesta. The one observable integumentary strand passes out between the outer and inner sarcotesta, more closely comparable with the position of the bundles in *Rhabdospermum* (Seward, '17, fig. 501E) than in *Cardiocarpus* (Seward, '17, fig. 500B). Moreover, the strikingly distinct integumentary tissues of *Rhabdospermum spinatum* compare very closely with *Rhabdospermum cyclocaryon* (see Brongniart, '81, pl. XII, fig. 3). The spinose nature of the sclerotesta of the new seed sets it apart from *R. cyclocaryon* or the apparently closely related *Mitrospermum compressum* (Will.) A. Arber.

Diagnosis:—Rhabdospermum spinatum: platyspermic seed 15 mm. broad x 12 mm. long; integument composed of four tissues: an outer conspicuously thick sarcotesta, inner sarcotesta, outer spinose sclerotesta, and inner sclerotesta of longitudinally elongate cells; pollen chamber shaped like an inverted shallow teacup.

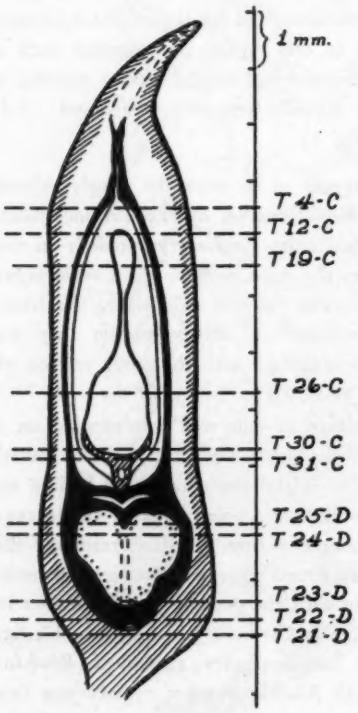
Locality: Urbandale Coal Mine, Des Moines, Iowa.

Horizon: Des Moines Series, Pennsylvanian.

Type specimen: No. WCB493, Henry Shaw School of Botany paleobotanical collections.

Kamaraspermum Leeanum Kern, gen. et sp. nov.

One of the Urbandale coal-balls received from Mr. Thompson contained a dozen or more specimens of a seed which, because of its highly distinctive structure, is designated herewith as a new species, *Kamaraspermum Leeanum*[2].



Text-fig. 3. *Kamaraspermum Leeanum.*

Diagrammatic median longitudinal section along the minor axis (from seed A, peel 475-T 21). Horizontal broken lines indicate corresponding positions of transverse sections through seeds C and D (see text-fig. 5). The seed tissues are indicated as follows:

Epidermis—outer black line.
Outer sclerotic integument—striped area.
Outer parenchymatous integument—white area.
Inner sclerotic integument—black.
Inner parenchymatous integument — white area delimited by inner sclerotic integument and thin black line.
Nucellus and megaspore membrane.
Probable course of vascular strand—broken line through basal chamber.
Parenchymatous cells of basal chamber—black dots.

[2]The prefix *Kamara* is from the Greek, meaning a chamber or room with an arched covering. The species is named for Mr. Arthur F. Lee, Chief Engineer of the Binkley Coal Company's Pyramid Mine, Pinckneyville, Illinois. Mr. Lee's most cordial cooperation during the past six years has been an indispensable aid in our coal ball collecting.

In spite of some pyritization the seeds are quite well preserved, and the orientation of two of them has allowed the preparation of satisfactory series of transverse and longitudinal peel preparations. The remaining specimens, although less completely preserved, have been useful in checking structures throughout.

The seed possesses an integument with a distinctive succession of tissues, and a large basal chamber, presumably a buoyancy mechanism which aided in water transport. The only previously described seeds with which it may be compared in a general way are those assigned to Brongniart's *Codonospermum*. However, the pronounced platyspermy of the Iowa seeds, as well as the nature of the integument, seems to render necessary a new generic name.

GENERAL ORGANIZATION—

The seed has the approximate shape of a double convex lens (pl. 19, figs. 4, 5), slightly elongated in the micropyle-peduncle axis, with an extended micropyle in the form of a flattened funnel. It measures about 12 mm. in length, and in a median transverse section the major and minor axes measure 11 and 3 mm. respectively. Thus, quite different aspects are presented, depending upon whether the longitudinal section is taken through the major (fig. 5) or minor (fig. 4) axis. In order to portray clearly the various aspects of the seed two sets of diagrammatic drawings have been prepared: one, from a series of transverse sections (text-fig. 4); and the other from a series of longitudinal sections (text-fig. 5)[3] taken through the minor axis.

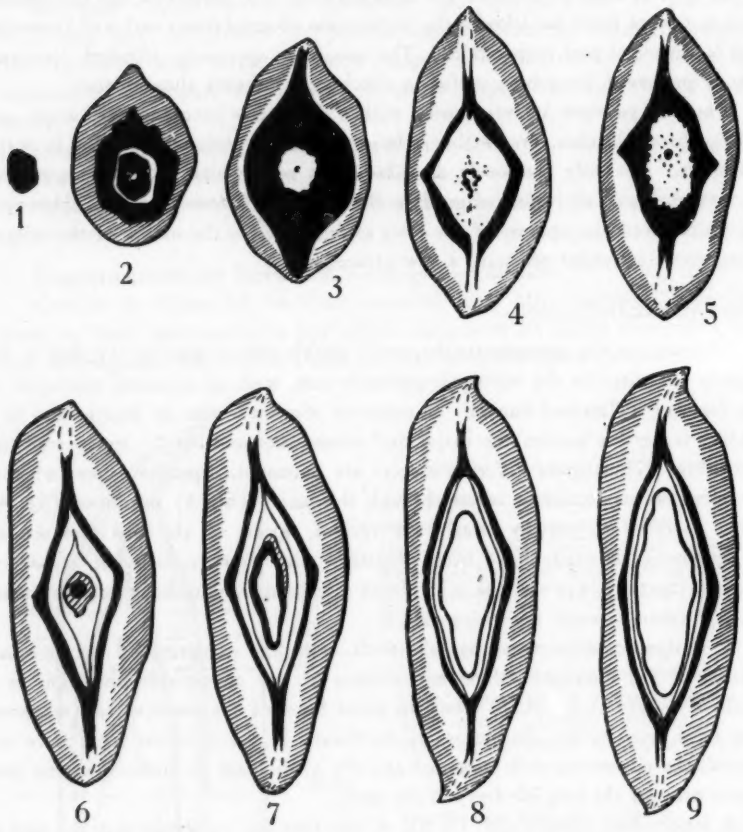
It is significant to note that the seeds, with the exception of the terminal portion of the micropyle, show no evidence of any appreciable distortion as a result of fossilization. Aside from the outer layer of the nucellus and whatever may have been within the megaspore membrane at the time the seeds were deposited, the tissues are well preserved and the gross shape as outlined in the text figures presents the true life form of the seed.

A longitudinal section (pl. 19, fig. 4, and text-fig. 3) shows that the seed is composed of two clearly defined regions: a basal chamber and nucellar chamber, with their attendant tissues. Simply as a matter of convenience these will be described separately.

NUCELLAR REGION—

Integument:—The integument is bounded externally by a single layer of epidermal cells which appear brick-shaped in both longitudinal and transverse sections. Within this epidermis is a succession of four clearly defined tissues. First

[3] All the seeds on which this description is based were contained in one small coal-ball specimen; thus it was not possible to prepare individual preparations of peels. Consequently in the longitudinal series shown in text-fig. 5 the preparations are slightly oblique, showing the micropyle or basal chamber more perfectly at one end than the other (cf. Nos. 2 and 3 with Nos. 15 and 16).



Text-fig. 4. *Kamaraspermum Leeanum*. A series of diagrammatic drawings of transverse sections extending from below the basal chamber to the top of the nucellar chamber.

1. Sclerotic tissue below basal chamber. Seed D, peel 475-T21.

2. Lowermost part of basal chamber containing prominent central vascular strand. Seed D, peel 475-T22.

3. Lower part of basal chamber. Seed D, peel 475-T23.

4. Upper part of basal chamber. Seed D, peel 475-T24.

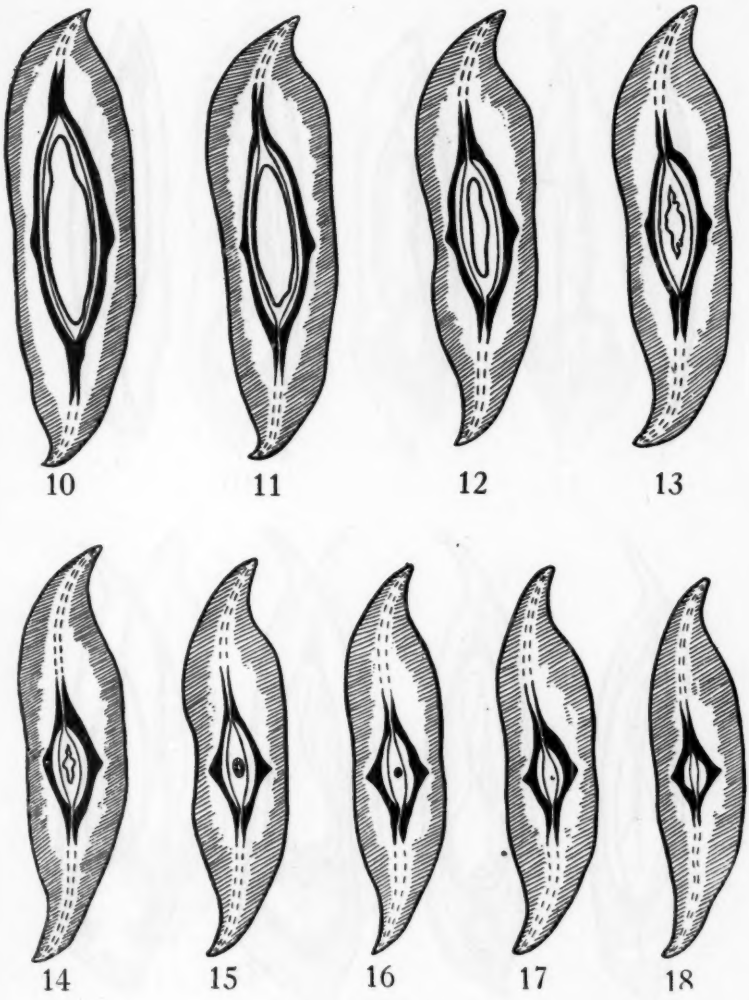
5. Top of basal chamber. Seed D, peel 475-T25.

6. Lower part of nucellar chamber, near nucellar attachment. This and the remaining figures in this series are from Seed C. Peel 475-T31.

7. Lower part of nucellar chamber. Peel 475-T30.

8. Lower third of nucellar chamber. Peel 475-T28.

9. Slightly below center of nucellar chamber. Peel 475-T26.

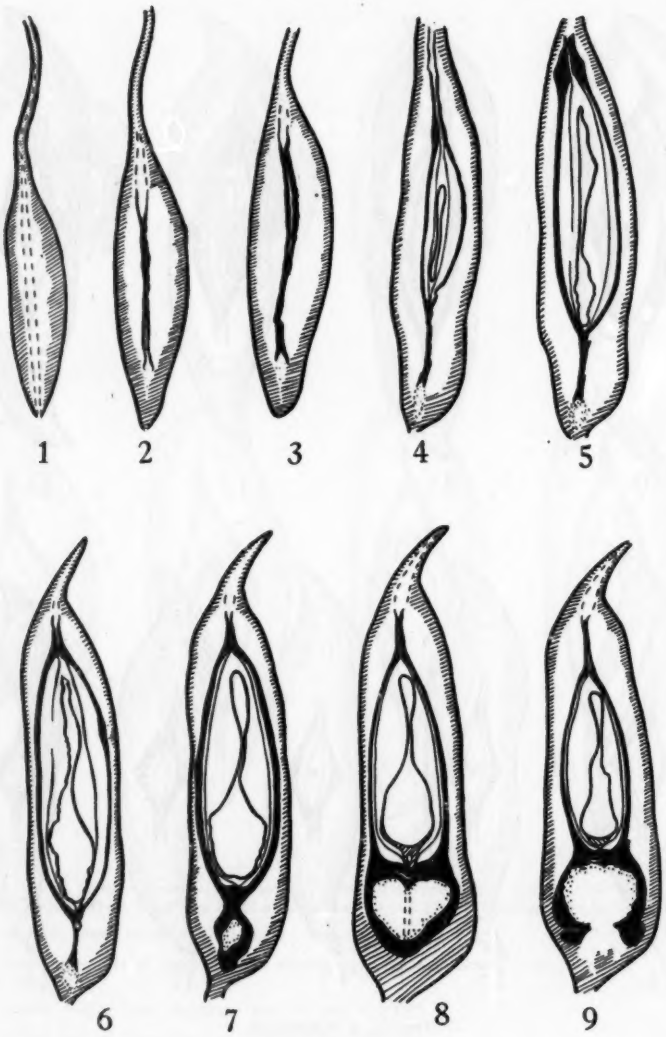


Text-fig. 4 (Continued).

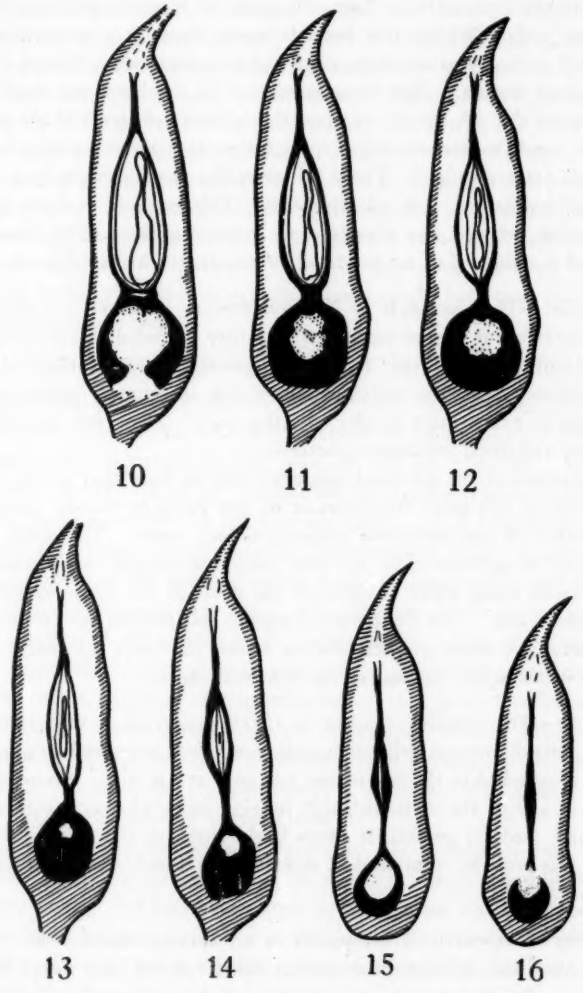
10. Slightly above center of chamber. Peel 475-T23.

11–16. From upper part of nucellar chamber. Peels 475-T21, T19, T18, T16, T15, T12 respectively.

17, 18. Top of nucellar chamber. Peels 475-T8 and T4.



Text-fig. 5. *Kamaraspermum Leeanum*. A series of diagrammatic drawings of longitudinal sections taken parallel to the minor axis of the seed. From seed A.



Text-fig. 5. (Continued).

1, peel 475-T33; 2, T32; 3, T31; 4, T28; 5, T26; 6, T24; 7, T23; 8, T21; 9, T19; 10, T18; 11, T16; 12, T13; 13, T11; 14, T7; 15, T5; 16, T2.

is a dark-colored layer, three or four cells thick, of rather large, irregularly shaped, thick-walled cells. Within this outer sclerotic tissue is a conspicuous layer of light-colored parenchymatous cells, small and hexagonal when viewed in transverse or longitudinal section. This tissue is massive in the lower micropyle region of the integument (pl. 19, fig. 4), tapering down rather abruptly in the mid-nucellar region, and tends to become slightly thicker in the transition zone between the nucellar and basal chambers. The third layer, like the outermost one, is relatively narrow and consists of dark sclerotic cells. This expands to form a very conspicuous tissue between the nucellar and basal chambers. The fourth layer is narrow, and is composed of longitudinally elongate, brick-shaped parenchyma cells.

Micropyle:—The micropyle of *Kamaraspermum Leeanum* is a striking structure and deserving of special comment. As may be noted in the median (minor axis) longitudinal section (pl. 19, fig. 4; text-fig. 3), it consists of two quite distinct regions: a massive continuation of the integument above the nucellar chamber, and a much more slender tapering apex. These will be referred to as the proximal and distal portions respectively.

The structure of the proximal micropyle differs from that of the integument below it only in the great development of the parenchymatous (second) layer, and an absence of the innermost parenchymatous tissue. The distal portion of the micropyle is approximately 1.5 mm. long and shaped like a much-flattened inverted funnel, being twice as broad in the plane of the major longitudinal axis as in the minor axis. Like the proximal region, this portion consists of epidermis, outer sclerotic and outer parenchymatous layers, the inner sclerotic layer having terminated in the upper portion of the proximal region.

Nucellus:—The nucellus consists of the remnants of a thin layer of small brick-like parenchymatous cells surrounding the well-preserved megaspore membrane, and is attached to the rest of the seed only at the base. No pollen chamber was found in any of the seeds, although judging from what is known of petrified Pennsylvanian seeds in general, it seems likely that one did exist. If such were the case it was probably composed of delicate cells which were destroyed prior to fossilization.

Megaspore membrane:—This appears as an orange-colored band immediately within the nucellus. It becomes somewhat thicker at the base where the nucellus is attached to the integument, and sometimes a few scattered cells may be seen within the membrane.

BASAL CHAMBER—

The basal chamber consists of epidermis, outer sclerotic and outer parenchymatous tissue, each identical to and continuous with the respective layers of the integument surrounding the nucellar chamber. An inner sclerotic layer surrounds the chamber and appears to be a continuation of the inner sclerotic integument

around the nucellar chamber. This layer consists of irregular cells, which are smaller than those of the outer sclerotic layer but resemble them in shape. The inner edge of this sclerotic layer is somewhat irregular, with a few small scattered parenchymatous cells occasionally adjoining it. Although not present in the specimens at hand, a vascular strand (indicated by the dotted line in text-fig. 3) probably passed from the peduncle through the center of the chamber. Evidence for it is an area of vascular tissue visible in cross-sections at the base and at the top of the chamber. Furthermore, in one of the median longitudinal sections the chamber is strongly heart-shaped due to the extension of some sclerotic cells downward from the top of the chamber. The fact that some scattered thin-walled cells are found within the chamber suggests that it might have been occupied with a loose aerenchymatous tissue.

COMPARISON WITH OTHER SEEDS—

Kamaraspermum presents a number of structural features that render it of very great interest, yet at the same time preclude it being assigned definitely to the recognized orders of Paleozoic seeds (Seward, '17, pp. 300–365). The strong bilateral symmetry and lack of any semblance of trigonocarpous organization in transverse section seem to rule out the Trigonocarpales. In at least three important respects it diverges from characteristic Lagenostomalean seeds: *Kamaraspermum* is strongly platyspermic; the nucellus is free from the integument; and the integument as a whole is comparatively thick. Its affinities lie closer to the Cardiocarpales than either of the previous two orders, and it seems most expedient to consider it tentatively as a member of this group. The chief conflicting feature here, however, is the structure of the integument. A typical Cardiocarp seed, as the present authors understand it, has an integument with a conspicuous and bulky outer fleshy sarcotesta, while *Kamaraspermum* presents in the sequence of its integumentary tissues: first (outermost), a sclerotic layer, then a relatively fleshy layer followed by another sclerotic one.

It is also appropriate to comment briefly on the prominent basal chamber. Usually where there is a lack of tissue in a petrifaction the possibility of loss through decay exists. Yet, since the Iowa seeds are generally well preserved and a similar basal chamber occurs in the French *Codonospermum* species (Brongniart, '74, '81; Renault, '96), it appears likely that the basal chamber existed as such in life. However, since the chamber region contains some remnants of delicate tissue it may be that it was occupied by a very loosely organized aerenchyma. In either event there can be little doubt that it functioned as a float mechanism.

The possibility of Lepidocarpalean affinities has also been considered. There are certain points of similarity between *Kamaraspermum* when viewed in median longitudinal section and a tangential section of a *Lepidocarpon* taken through the "heel" or distal end of the sporophyll. The symmetry of *Kamaraspermum*, its complex integument and micropyle, nature of the remnants of the outer nucellar

tissue are, upon more critical examination, found to be in no way related to those of any described species of *Lepidocarpon*.

One is almost tempted to apply the nebulous term "missing link" to this curious fossil, with its non-conformity to established groups, yet the evidence seems to point in the direction of the Cardiocarpales, to which order it is tentatively assigned.

Diagnosis:—Kamaraspermum Leeanum: platyspermic seed 12 mm. long, 11 x 3 mm. in median transverse section; conspicuous chamber beneath nucellar region; integument composed of thin outer sclerotic layer, conspicuous fleshy layer, inner sclerotic layer, and thin inner parenchymatous tissue; micropyle of two clearly defined regions: a massive continuation of integument above nucellar chamber, and delicate distal portion shaped like a flattened funnel.

Locality: Urbandale Coal Mine, Des Moines, Iowa.

Horizon: Des Moines Series, Pennsylvanian.

Type specimen: No. WCB475, Henry Shaw School of Botany paleobotanical collections.

Literature Cited:—

Arber, A. (1910). On the structure of the Palaeozoic seed *Mitrospermum compressum* (Will.). Ann. Bot. **24**:491–509.

Arnold, C. A. (1938). Paleozoic seeds. Bot. Rev. **4**:205–234.

Brongniart, A. (1874). Études sur les graines fossiles trouvées à l'état silicifié dans le terrain Houiller de Saint-Étienne. Ann. Sci. Nat. Bot. V. **20**:234–265 (see p. 257).

———, (1881). Recherches sur les graines fossiles silicifiées. Paris.

Graham, R. (1934). Pennsylvanian flora of Illinois as revealed in coal balls. I. Bot. Gaz. **95**:453–476.

Krick, H. V. (1932). Structure of seedlike fructifications found in coal balls from Harrisburg, Illinois. *Ibid.* **93**:151–172.

Oliver, F. W. and E. J. Salisbury. (1911). On the structure and affinities of the Palaeozoic seeds of the *Conostoma* group. Ann. Bot. **25**:1–50.

Rénault, B. (1896). Étude des gites minéraux de la France. Bassin Houiller et Permien d'Autun et d'Epinac. Flore fossile. Paris.

Seward, A. C. (1917). Fossil plants. III. Cambridge.

Williamson, W. C. (1877). On the organization of the fossil plants of the Coal Measures. pt. viii. Phil. Trans. Roy. Soc. London **167**:213–271.

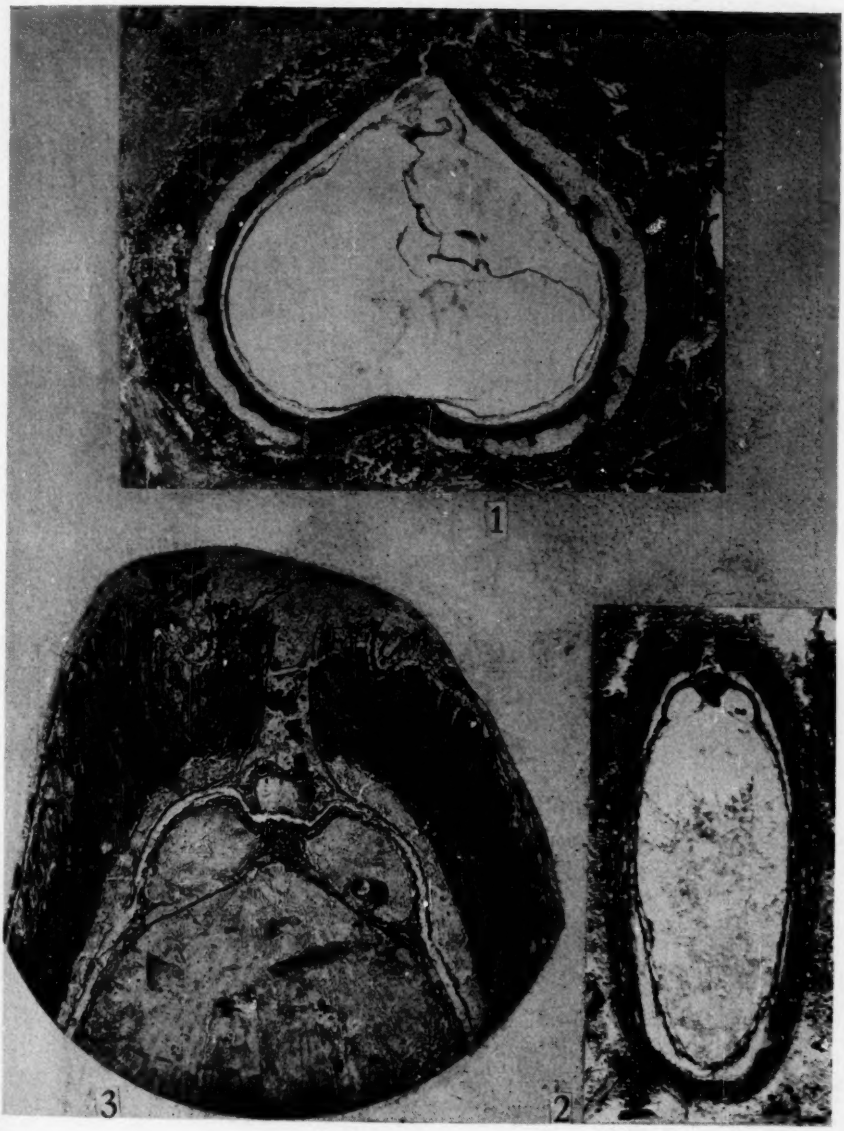
Explanation of Plate

PLATE 18

Fig. 1. *Rhabdospermum spinatum*. A nearly median longitudinal section. See text-fig. 2 for cellular structure of integument. From peel 493-T3. Magnified x 6.

Fig. 2. *Conostoma oblongum* Williamson. A nearly median longitudinal section through the seed. From slide 1398. Magnified x 13.5.

Fig. 3. The micropylar region of the seed shown in fig. 2, at a higher magnification. From slide 1398. Magnified x 41.



KERN & ANDREWS—AMERICAN CARBONIFEROUS FLORAS. IX

Explanation of Plate

PLATE 19

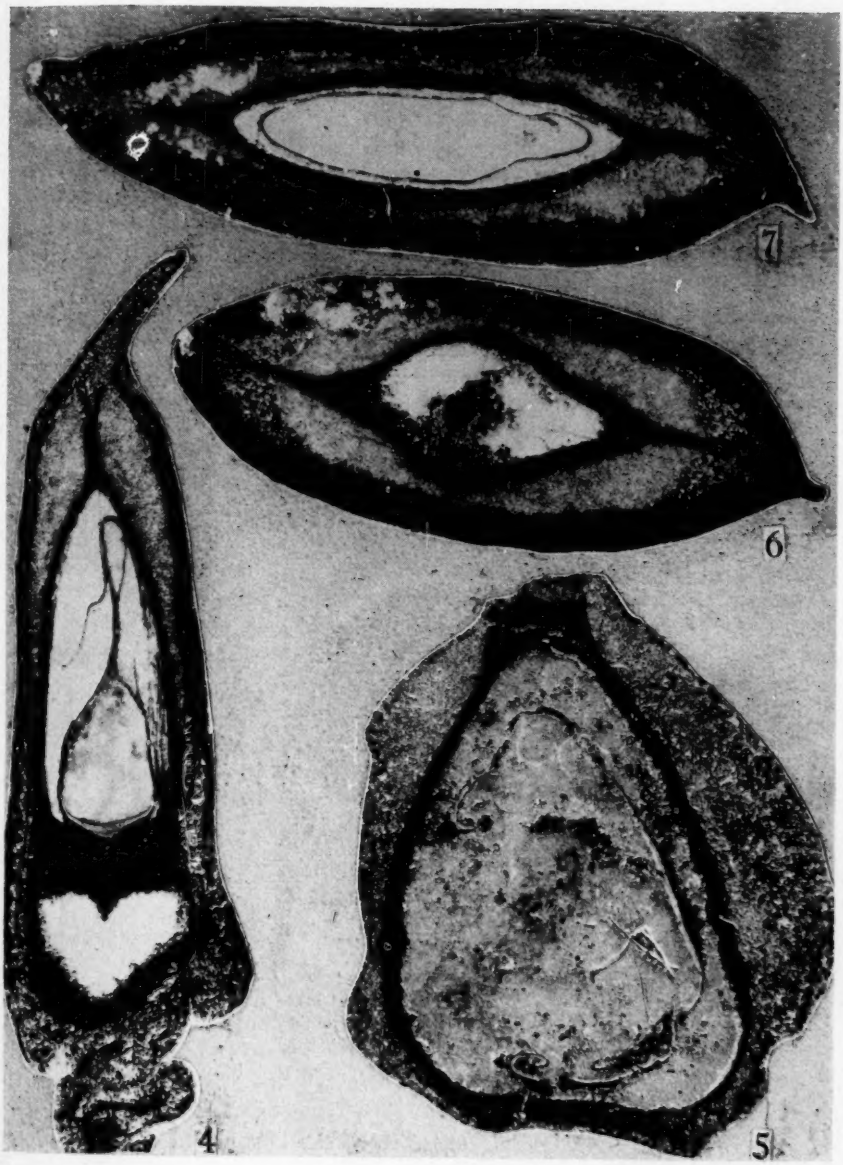
Kamaraspermum Leeanum Kern

Fig. 4. Median longitudinal section along minor axis. Seed A, peel 475-T21. x13.

Fig. 5. Longitudinal section along major axis. Peel 475-T14. x 12.

Fig. 6. Transverse section through lower part of nucellar chamber, just above point of nucellar attachment. Seed C, peel 475-T26. x 12.

Fig. 7. Transverse section through central portion of nucellar chamber. Seed C, peel 475-T23. x 12.



KERN & ANDREWS—AMERICAN CARBONIFEROUS FLORAS. IX

THE GALLATIN FOSSIL FOREST

HENRY N. ANDREWS AND LEE W. LENZ

The petrified forests of Yellowstone National Park have been a source of wonder ever since the early explorations of Jim Bridger, and that wonder grew into a more realistic admiration of geologic time and the forces that preserve plants through the ages following the studies of Dr. F. H. Knowlton during the latter years of the last century. In spite of the fact that some of the plant names in Knowlton's monograph would probably meet with revision in the hands of a present-day paleobotanist of Tertiary floras, it is likely that it will always stand as a classic in the annals of the science. It brought to light a flora, or more correctly a series of floras, strikingly different from the one that exists there to-day, and it made generally known the most spectacular of all petrified forests.

Fossil forests representing diverse ages in the earth's history, various modes of preservation, and a wide range of plant groups have been acclaimed by numerous authors. Perhaps the best known of all are the petrified trees of Arizona, although there the wood is, for the most part, too highly replaced to be of botanical value, and the great trees were transported some distance from their original habitat prior to petrification. Certainly among the most unique fossil floras, from the standpoint of the plants themselves, is the Devonian *Eospermatopteris* deposit near Gilboa, New York, and the Jurassic Cycad forest of the Black Hills. Unfortunately there is but little to be seen of these in the field. Through the work of the New York State Museum the "Dawn-seed-ferns" of Gilboa have been made to live again in an admirably executed restoration, and one may catch a glimpse of one of the earliest forests that existed on the earth. And we may be consoled in the knowledge that a large and representative series of the Dakota Cycads rests in security through the vigorous collecting activities of Professor Wieland. The same author has also given us a picture of the great Patagonian forest, especially remarkable for the prodigious abundance of petrified Araucarian cones that it has yielded.

In the coal balls and shales of the Carboniferous there is ample evidence of the Pteridophytic and early seed-plant forests that once covered so much of the globe, while occasionally, as with the Lycopod stumps preserved in Victoria Park, Glasgow, we see fragments of the forests in place. Another remarkable forest, preserved in a more precise sense of the word, is the one at Florissant, Colorado. Here a profusion of foliar remains, along with occasional representatives of the animal population, are preserved in volcanic ash beds immediately surrounding the stumps which were petrified in their original position in life. This is a rarely enough encountered combination of the trees and foliage that they bore. The impressions have been treated by a number of authors and are deserving of a comprehensive revision.

The discovery, in some abundance, of the silicified trunks of the Cretaceous *Tempskya* tree ferns in various of the northwestern states, and especially Idaho, has revealed a widespread and unique forest tree. But, like the Arizona petrifactions and numerous other western fossil wood deposits, these are not found in their original place of growth and hardly deserving of the term "fossil forest."

All of these forest remains of the past are important and distinctive in their own way and the age that they represent. Yet none of them can vie with the immense grandeur in both space and time of the Yellowstone forests. Nowhere else does there exist the succession of one forest directly above the grave of its predecessor—a succession that emphasizes perhaps more forcefully than any other plant fossil deposit the immensity of geologic time. Individually these forests attained ages well exceeding 1,000 years, and there is a minimum of no less than 16 of them extending one above the other.

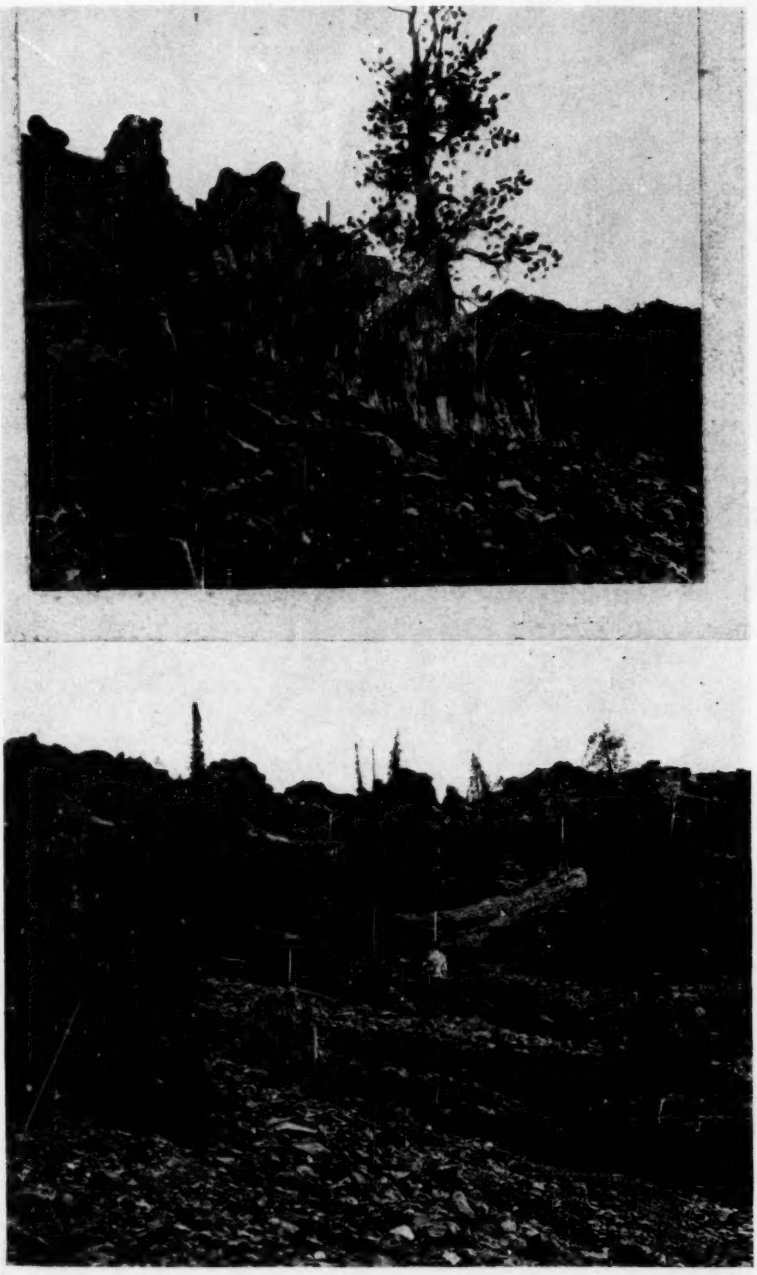
The petrified forests in the Specimen Ridge region south of the Lamar River, being not far distant from the main stream of travel through the Park, are rather well known. It is the purpose of this article to focus some attention on the fine display of fossil forests in the extreme northwest corner of the Park. Although this is a region that is by no means unknown, it is certainly worth more attention than it has received from botanists and geologists, whether amateur or professional.

There is a fine camp ground about 300 yards up Specimen Creek from the Gallatin Canyon highway (Route U. S. 191), and shortly to the north there is noted a "Fossil Forest" on the U. S. Geological Survey's map of the Park. From our own observations the finest exposure of the forests is found about two miles northeast of this point considerably closer to the summit of Big Horn Peak. While it is quite possible to make the climb, see a good deal of the forests, and return to the camp ground on the same day, a two-day trip allows a more leisurely and profitable study.

An excellent trail leaves the highway and follows along the north side of Specimen Creek. Two unnamed tributaries may be noted on the topographic map flowing in from the north, the second of which departs from Specimen Creek about one and three-quarters miles from the camp ground. About a quarter of a mile north of the trail this divides into two branches which, for the sake of clarity, may be referred to as the West Fork and East Fork, although no names are designated on the map. On our trip of last summer we packed in provisions for overnight and set up camp about 200 yards north of the point where the two forks join.

The finest succession of fossil forests that were encountered occur on the southwest slope of the spur on either side of which the two forks flow. In ascending this spur petrified stumps were found at about the 8,000-foot contour, and splendid displays of at least ten successive forests were counted extending up the rocky exposed southwest face of the spur.

It is well to emphasize that figures given here are only approximate inasmuch as surveying instruments were not employed, and the number of forests given is a very conservative minimum. A "forest" was recorded only where a series of at



Upper: A petrified stump of *Sequoia magnifica* Knowlton, 14 feet in diameter.
Lower: A portion of the Gallatin fossil forest showing silicified stumps at three successive forest levels.

Upper: An exceptionally tall specimen in the Gallatin fossil forest. Most of the trees weather to within a few feet of the surrounding breccias.
Lower: Two stumps showing roots intact.

least four or five stumps could be traced along the same horizon, or where rooted specimens were observed. A more precise counting of the forests was hampered by two factors—the cover of modern vegetation on the lower slopes of the spur as well as the upper reaches on and immediately below the ridge, and the nearly precipitous nature of the terrain above the uppermost reaches of the West Fork, as is clearly indicated on the topographic sheet of the Park.

Modern vegetation covers the volcanics at about the 8,500-foot contour of the spur, and it is necessary to follow along in a westerly direction almost to the Left Fork. The breccias are well exposed in the stream bed and its immediate vicinity and above its uppermost limit for another 500 feet or more. Here six more forests were defined at less regular intervals. It is almost certain that the actual number preserved here must be at least twice that recorded, but due to the steepness of the slope most of the stumps do not remain long in position once they start to weather out.

The average vertical distance between the ten successive forests that were counted on the spur slope was about 25 feet, with a variation of about 15 to 35 feet. To determine the distance more exactly between forests would require leveling instruments and considerable excavation inasmuch as only occasionally are the stumps exposed to the roots. Since the area is most unique and a National Park as well, the latter treatment would hardly be justified. Thus, while evidences of sixteen successive eras of forest growth were found on the spur and the upper reaches of the Left Fork ravine it seems safe to suggest that half again that number would be revealed by more detailed study. Such evidence is hardly required to emphasize the spectacular nature of the forests.

On the second day we ascended the spur that lies between the two previously mentioned tributaries of Specimen Creek. The fossil forests are first met at a somewhat higher level here partly because the living vegetation cover extends up higher, and partly because the beds dip toward the southeast. Following this spur to about the 8,300-foot contour one may then traverse about 100 yards to the west into an exceedingly rugged ravine where numerous stumps are exposed through a vertical distance of some few hundreds of feet. The successive forests cannot be traced as clearly in this sector although it is of interest for the large size of some of the stumps, a Sequoia 14 feet in diameter being the largest that we encountered. Although a central core some 5 feet in diameter had been destroyed in this tree prior to fossilization a study of wood specimens from the remaining part of the trunk showed an average of 19 rings to the inch, indicating an age of about 1,600 years for the tree.

Unfortunately there were few evidences of well-preserved foliar remains in the territory that was covered. The forests do extend for some distance to the northwest, however, and it is possible that leaf impressions might be found at other points. If such were located the possibility of an ecological study is evident and should produce most interesting results. Aside from this the region is well worth a day's time for any naturalist with a paleontological bent.